Journal of World's Poultry Research

BOOKLET

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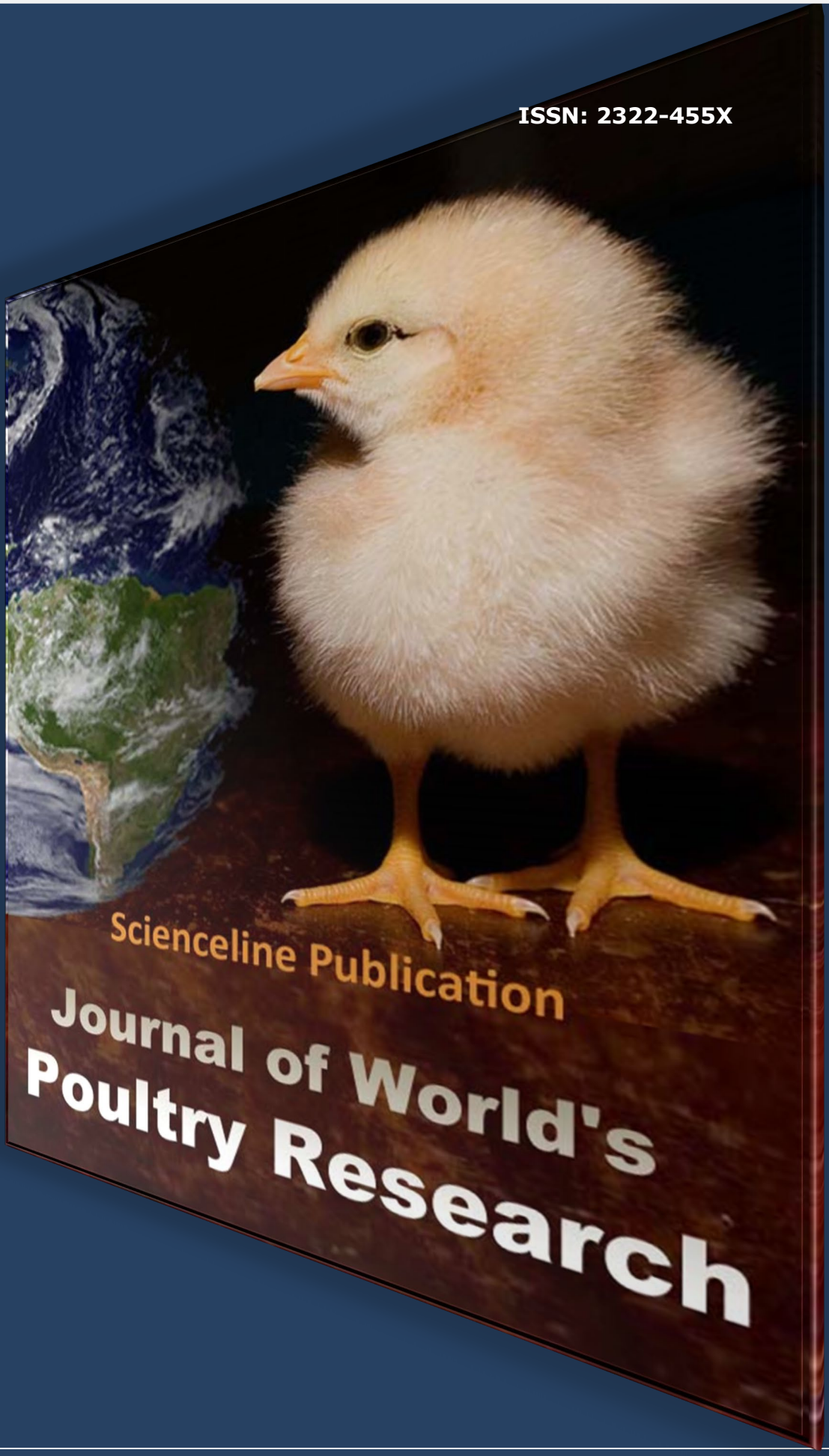

Volume 12, Issue 3, September 2022

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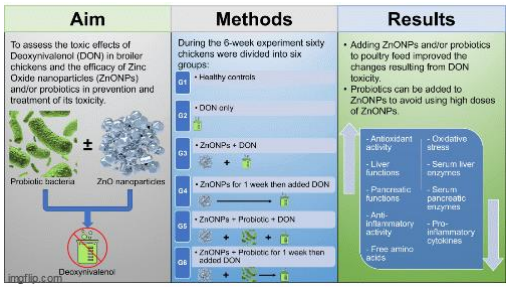
Volume 12 (3); September 25, 2022

Research Paper

Controlling Immunomodulation Effects of Deoxynivalenol Mycotoxins by NanoZinc Oxide and Probiotic in Broiler Chickens

Sayed-ElAhl RMH, Hassan AA, Mogda K Mansour, Abdelmoteleb AMM, and El-Hamaky AMA.

J. World Poult. Res. 12(3): 133-141, 2022; pii: S2322455X2200015-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.15



ABSTRACT: The elimination of adverse toxic effects of mycotoxins is currently the main strategy in animal production, particularly in poultry. The current study investigated the influence of chronic administration of deoxynivalenol on the health status, biochemical and immunological parameters of broiler chickens and the efficacy of ZnO-NPs and probiotics in preventing and treating the effect of toxicity. The experiment program lasted 6 weeks and was performed on a total of 60 broiler chickens aged 5 days, divided into six groups. Group 1 received healthy feed free of toxins, group 2 was fed with deoxynivalenol (DON), group 3 received Zinc Oxide nanoparticles (ZnO-NPs) and DON, group 4 had ZnO-NPs for 1 week, then DON was added for the remaining 5 weeks, group 5 was fed on ZnO-NPs, 1 g probiotic powder/kg of diet, and DON, group 6 had ZnO-NPs and 1 g probiotic powder/kg of diet for 1 week, then DON was added for 5 weeks. The used dose of ZnO-NPs was 50 ppm, and DON was 5 ppm in the diet. The intoxicated chickens showed adverse changes as increased pro-inflammatory cytokines, serum hepatic, and pancreatic enzymes, as well as decreased free amino acids. The supplementation of ZnO-NPs and/or probiotics improved all toxic changes resulting from DON toxicity, indicating that the metal nanoparticles and probiotics can be used together in poultry feed to avoid the addition of high doses of ZnO-NPs. Therefore, the use of 50 ppm of nanomaterial supplementation plus 1 g probiotic/ kg feed for the degradation of mycotoxins in poultry feed is recommended as it is safe and affordable.

Keywords: Deoxynivalenol, Fusarium spp., Nanoparticles, Poultry, Probiotic

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Research Paper

A Microscopic Study on Morphology of Reactive Thrombocytes in Duckling

Cotter PF.

J. World Poult. Res. 12(3): 142-150, 2022; pii: S2322455X2200016-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.16

ABSTRACT: Thrombocytes, well known as important clotting factors, and now known to be important as phagocytic cells, might benefit the study of the avian hemogram. Therefore, blood was sampled from 4 late-stage embryos at embryo day 24, and 4 one-day-old hatchlings (d1), and 5 female ducks aged 59 weeks stained by Wright-Giemsa and examined at 100x. Standard differential counts (SDC) of 2 x 200 cells were used to determine total white blood counts (TWBC) and heterophil/lymphocyte (H/L) ratios. Thrombocytes were not included in the SDC but were studied from photomicrographs. Reactive thrombocytes were present in blood films having a normal TWBC or in the presence of leukocytosis (59 weeks). The H/L ratios may or may not be elevated. Reactive thrombocytes can be differentiated from quiescent types on morphologic criteria. These included an increase in the number of magenta "specific granules", the development of cytoplasmic vacuoles, and a capacity to form aggregates with other Th or with cells of another series. Reactive Th were not necessarily larger in size than quiescent types. In some instances, Th aggregation with RBC (toroid formation) was with sufficient force to distort the RBC cell membrane. It was observed that reactive thrombocytes were accompanied by bacteria, either free-swimming or attached to cell-associated bacteria. Reactive thrombocytes having lost portions of their cell membrane were regularly encountered. As avian thrombocytes

are now recognized as important phagocytic cells, as well as having a primary role in hemostasis, they are part of the immune defense mechanism. The presence of reactive thrombocytes in a hemogram should be considered when using hematological data to evaluate immune responses and establish stress status.
Keywords: Hematology, Immunity, Reactive thrombocyte, Simple and complex toroid, Stress

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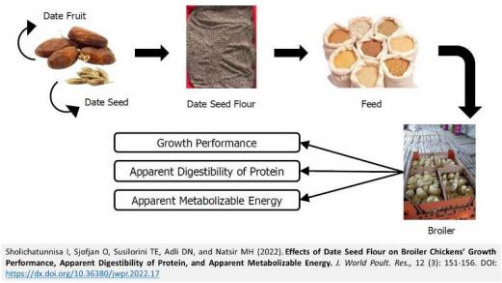
Research Paper

Effects of Date Seed Flour on Broiler Chickens' Growth Performance, Apparent Digestibility of Protein, and Apparent Metabolizable Energy

Sholichatunnisa I, Sjofjan O, Susilorini TE, Adli DN, and Natsir MH.

J. World Poult. Res. 12(3): 151-156, 2022; pii: S2322455X2200017-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.17

ABSTRACT: The use of agricultural by-products as alternative feed ingredients is recommended to reduce production costs and maximize income. This study aimed to determine the effects of added date seed flour on broiler chickens' growth performance, protein digestibility, and metabolic energy. A total of 200 Lohmann MB-202 day-old chicks were randomly allocated to 5 treatments and 4 replication with 10 broiler chickens per cage. The treatments used in the current research included control without the addition of date seed flour (T0), basal feed + 2.5% date seed flour (T1), basal feed + 5.0% date seed flour (T2), basal feed + 7.5% date seed flour (T3), basal feed + 10% date seed flour (T4). The investigated parameters were growth performance, apparent digestibility of protein, and apparent metabolizable energy-nitrogen (AMEn). The result showed that adding date seed flour significantly affected final body weight, apparent digestibility of protein, and AMEn. In contrast, the date seed flour was no significant effect on the feed intake feed conversion ratio and income over feed cost. In conclusion, the addition of 10% date seed flour successfully increases final body weight, apparent digestibility of protein, and AMEn without any adverse effect on the broiler chickens.
Keywords: Broiler chicken, Date seed flour, Metabolizable energy, Performance, Protein digestibility



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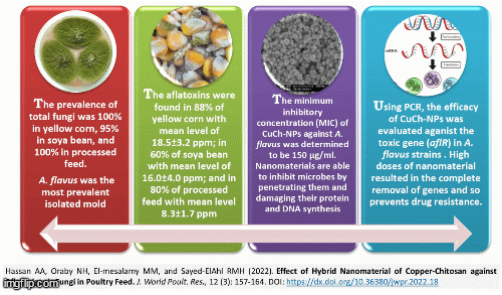
Research Paper

Effect of Hybrid Nanomaterial of Copper-Chitosan against Aflatoxigenic Fungi in Poultry Feed

Hassan AA, Oraby NH, El-mesalamy MM, and Sayed-ElAhl RMH.

J. World Poult. Res. 12(3): 157-164, 2021; pii: S2322455X2200018-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.18

ABSTRACT: In the past decades, the application of nanotechnology indicated significant improvements in animal health. In the present work, 60 samples of poultry feeds were examined, including 20 samples for each yellow corn, soya bean, and processed feed. The prevalence of total fungi was reported as 100%, 95%, and 100% in yellow corn, soya bean, and processed feed, respectively. Toxin-producing *Aspergillus flavus* represented 75% of isolates from yellow corn, 88% from soya bean meal, and 50% from processed feed. Aflatoxins were found in 88%, 60%, and 80% of yellow corn, soya bean, and processed feed with mean levels of 18.5 ± 3.216.0 ± 4.08.3 ± 1.7 ppm, respectively. The copper nanoparticles embedded with chitosan were green synthesized using an eco-friendly method, and their antifungal activity was evaluated against aflatoxigenic mold that recovered from poultry feeds. However, the molecular detection of virulent genes of *Aspergillus flavus* (*aflR* gene*)* after their exposure to high doses of copper-chitosan nanoparticles (CuCh-NPs) 150 µg/ml prevents *aflR* gene expression. The embedded chitosan with copper nanomaterial helps decrease their suspected toxicity to animals by reducing the used doses. Hence, the use of nanocomposites of nanomaterials with green benefits substances, such as chitosan, was the essential strategy of field application in veterinary.
Keywords: *Aspergillus flavus*, Chitosan, Copper nanoparticles, Nanotechnology, Poultry feed



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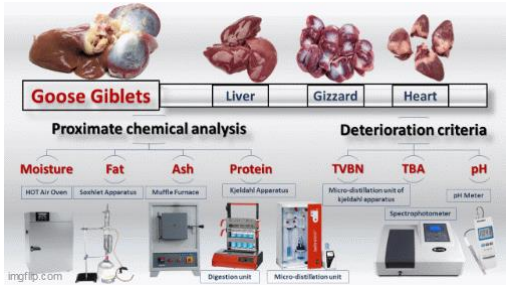
Research Paper

Proximate Chemical Analysis and Deterioration Criteria of Goose Giblets

Nagy ZM, Emara MMT, Yessien NA, and Zaki HMBA.

J. World Poult. Res. 12(3): 165-170, 2022; pii: S2322455X2200019-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.19



ABSTRACT: Goose meat is one of the most common types of meat consumed worldwide. Egyptian goose species, known as *Alopochen aegyptiacus* is one of the first reared poultry species. As meat consumption and the need for animal protein rise globally, edible giblets can serve as abundant protein and fat sources. Recently, edible giblets have become readily available, quick-to-prepare food on the market. This study aimed to reveal the proximate chemical composition (protein, fat, moisture, and ash) as well as the deterioration criteria (pH, Total volatile basic nitrogen [TVBN] value, and thiobarbituric acid reactive substance [TBA] value) of Egyptian goose giblets, including liver, gizzard, and heart. A total of 60 samples of goose giblets, including liver, gizzard, and heart (n = 20 each), were collected from Giza and Cairo cities, Egypt. The results showed a marked variation among each giblet type. The goose's highest protein content (24.48%), moisture content (72.42%), and fat content (12.18%) were recorded for liver, gizzard, and heart, respectively. Moreover, the highest pH (6.72) and TVBN mean value (5.61 mg/100 gm) were indicated in goose's livers, while the highest TBA mean (0.67 mg malonaldehyde/kg) was obtained from goose' hearts. These findings may provide a clear understanding for both consumers and possessors about the nutritional value of goose giblets which could be used as an alternative protein source. Moreover, the obtained data in the current study could help meat technology processors to add nutritional value to goose products using goose giblets.
Keywords: Chemical analysis, Deterioration criteria, Fat, Giblets, Goose, Protein, pH

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Research Paper

The Impacts of Locally Cultivated Herbs on Physical Parameters and Meat Quality of Broiler Chickens

Al Hanna R.

J. World Poult. Res. 12(3): 171-180, 2022; pii: S2322455X2200020-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.20

ABSTRACT: Herbs greatly influence broiler chickens' performance and may alternate the use of antibiotics in the poultry sector. The study investigated the effects of local natural herbs on Ross 308 broiler chickens' physical characteristics and meat quality. A total of 702-day-old broiler chickens were divided into two trials consisting of 13 treatment groups fed 11 diets. Treatments included control groups (CGIa and CGIb); Basal diet (BD) free of antioxidants and antibiotics, and CGIIa, CGIIb; BD complemented with antioxidants and antibiotics, and experimental groups (EG); EGIII (1% peppermint + BD), EGIV (1% thyme + BD), EGV (0.5% peppermint + 0.5% thyme), EGVI (1% rosemary + BD), EGVII (1% chamomile flowers + BD), EGVIII (0.5% rosemary + 0.5% chamomile), EGIX (1% onion powder + BD), EGX (1% garlic powder + BD), and EGXI (0.5% onion powder + 0.5% garlic powder). Maximum and minimum feed intake averages were in EGV and EGIII (94.90 and 77.74 kg/group, respectively). Live body weight gains of both EGVI and CGIIa were significantly higher than EGIV, EGVIII, and CGIa. Chicks of EGVIII showed the lowest net weight percentage in relation to live body weight (carcass yield). Breast meat pH ranged between 5.26 and 6.14 (in EGVII and EGIV, respectively) 24 hours after cooling, and between 5.86 and 6.11 (in CGIa and EGVI, respectively) one month after freezing. Breast meat lightness was significantly higher in EGVI than EGVIII at 24 hours after cooling, and it was the highest in EGVI 1 month after freezing. Breast meat redness was the highest in CGIIa at 24 hours after cooling. EGIX showed a significantly higher redness value one month after freezing than EG X, CG Ib, and CG IIb. Yellowness ranged between 7.58 and 13.54 for EGX and CGIa, respectively, 24 hours after cooling, and between 8.29 and 13.95 a month after cooling for EGX and EGV, respectively. Tested herbs had comparable effects to antibiotics on chicken growth and meat quality. Rosemary (1%) had an ameliorative effect on chickens' body growth. Chamomile (1%) as well as thyme and peppermint mix (0.5% each), improved the palatability for feed.
Keywords: Broiler chicken, Chamomile, Garlic powder, Onion powder, Peppermint, Rosemary, Thyme

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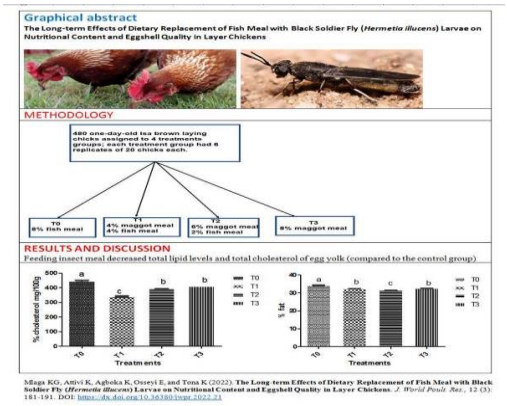
Research Paper

The Long-term Effects of Dietary Replacement of Fish Meal with Black Soldier Fly (*Hermetia illucens*) Larvae on Nutritional Content and Eggshell Quality in Layer Chickens

Mlaga KG, Attivi K, Agboka K, Osseyi E, and Tona K.

J. World Poult. Res. 12(3): 181-191, 2022; pii: S2322455X2200021-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.21



ABSTRACT: Although the egg is one of the foods offering nutrients of high biological value, the diet of layer chickens can change these characteristics. The aim of this study was to evaluate the effect of a long-term dietary replacement of fish meal with maggot meal of black soldier fly larvae on egg quality of hens. A total of 480 one-day-old Isa brown chicks were randomly assigned to 4 dietary treatment groups. The groups were named T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal), and T3 (8% maggot meal). Each treatment group had 6 replicates of 20 chicks each. Data were collected on the eggshell quality parameters between 22 and 56 weeks of age. The results indicated that egg weight, shell weight, shape index, shell index, egg surface area, egg volume, density, yolk pH, albumen pH, yolk and albumen moisture content, yolk color, and yolk height were not influenced by the use of larval meals. Although the proportion of the yolk increased with age, there was no interaction between the use of fly larvae and the duration of its use for the collected parameters. However, the proportion of albumen, Haugh's unit in T1 and T3 treatments were higher than those of T0 and T2. The proportion of egg yolk, the yolk to albumen ratio, and the count of cracked eggs of T0 and T2 varied significantly compared to T1 and T3. Total egg fat decreased significantly as a result of the use of maggot meal. Total cholesterol, High-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol, and LDL/HDL ratio were lower in groups fed larvae meal, compared to the control group. It was concluded that the use of black soldier fly larvae meal during the entire rearing cycle and period of layers did not adversely affect the eggshell quality and nutritional content of the eggs.
Keywords: Black soldier fly, Cholesterol, Egg quality, Haugh unit, Larvae meal, Lipid

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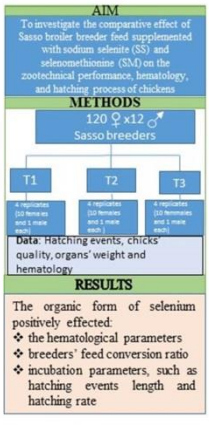
Research Paper

Effect of Selenium-based Diets on Zootechnical Performance, Hematological Parameters, and Relative Weight of Internal Organs in Broiler Chickens

Tona K, Nenonene AY, Fiougou S, Oke E, Fafiolu AO, and Pitala W.

J. World Poult. Res. 12(3): 192-198, 2022; pii: S2322455X2200022-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.22



ABSTRACT: Two sources of selenium are commonly used in poultry nutrition, the organic and the inorganic forms. This study was carried out to investigate the comparative effect of Sasso broiler breeder feed supplemented with sodium selenite (SS) and selenomethionine (SM) on the zootechnical performance, hematology, and hatching process of chickens. A total of 120 female broiler breeders and 12 roosters of Sasso strain at 47 weeks were equally assigned to three treatments with four replicates per each, including 10 breeders crossed with 1 rooster. The treatment groups were broiler breeders fed a basal diet without selenium supplementation (control), chickens fed the basal diet supplemented with SS, and breeders fed the basal diet supplemented with SM. The inclusion level of each selenium was 0.2 ppm. The collected data included feed intake and egg weight during 8 weeks. In the end, blood samples were collected for hematological investigations. A total of 150 hatching eggs were collected from each treatment. After recording their weight, the eggs were incubated at adequate temperature and relative humidity. On day 18 of incubation, the eggs were weight again, candled, and transferred into the hatcher. Each egg was individually checked every 3 hours during the last 3 days of incubation for hatching events determination. The results showed that breeders fed SM had the lowest feed conversion ratio. There was an increase in the majority of blood parameters in breeders fed SM, compared to other treatments. The lowest duration of the hatching events was observed with breeders fed SM, and consequently, they had the best

hatching rate but without any significant difference in the chicks' quality and their weight of internal organs at the hatch. In conclusion, this study demonstrated that using selenium is beneficial, especially in the organic form, which appeared to be more efficient, compared to the inorganic form.
Keywords: Broiler Chicken, Hatching events, Hematology, Selenomethionine, Sodium selenite

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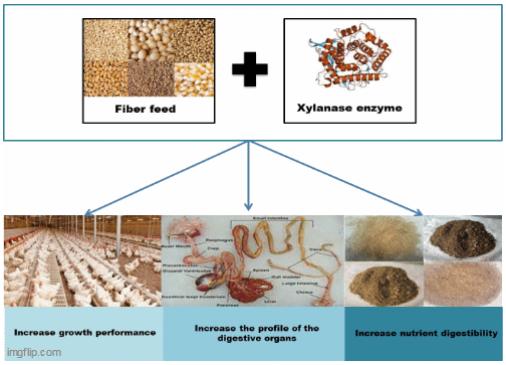
Review

Effects of Xylanase Supplementation on the Performance, Nutrient Digestibility, and Digestive Organ Profiles of Broiler Chickens: A Meta-analysis

Inayah SR, Mutia R, Jayanegara A, Yanza YR, and Amnah S.

J. World Poult. Res. 12(3): 199-211, 2022; pii: S2322455X2200023-12
DOI: https://dx.doi.org/10.36380/jwpr.2022.23

ABSTRACT: Enzymes supplementation in broiler feeding is commonly applied to optimize animal feed utilization and reduce feed production costs. One of the enzymes widely used in the broiler industry is xylanase which breaks down complex fibrous compounds in feed, such as nonstarch polysaccharides, to simpler utilizable sugar molecules. However, the effects of xylanase enzymes on broiler growth performance, nutrient digestibility, and organ function in broiler chickens were variable and inconclusive. Therefore, the current study aimed to evaluate the effect of the xylanase enzyme in feed on the performance, nutrient digestibility, and digestive function of parrots using a meta-analysis approach. A dataset of 140 points obtained from 53 articles was analyzed using a mixed model methodology. The results showed that the xylanase enzyme supplementation increased the broiler's body weight gain and decreased feed consumption and feed conversion ratio. In addition, xylanase supplementation also increased nutrient digestibility, such as dry matter, crude protein, starch, gross energy, fat, phosphorus, and calcium. Concerning broiler organ weights, the xylanase supplementation in broiler feed significantly reduced the weight of the duodenum, small intestine, and relative length of the duodenum, jejunum, and ileum. Xylanase supplementation also tended to reduce the relative weight of the proventriculus. The results also showed a negative response to the crypt depth ileum of broiler due to xylanase supplementation. It can be concluded that xylanase supplementation improves the performance, nutrient digestibility, and digestive function of broiler chickens.



Keywords: Broiler chickens, Nonstarch polysaccharide, Performance, Xylanase enzyme

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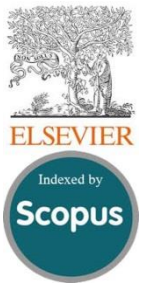

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DOI: https://dx.doi.org/10.36380/jwpr.2022.15

Controlling Immunomodulation Effects of Deoxynivalenol Mycotoxins by NanoZinc Oxide and Probiotic in Broiler Chickens

Rasha Mahmoud Hamza Sayed-ElAhl[1]*, Atef Abdelaziz Hassan[1], Mogda Kamel Mansour[2], Azza Moustaffa Moustaffa Abdelmoteleb[2], and Ahmed Mohamed Attia El-Hamaky[1]

[1]*Department of Mycology and Mycotoxins, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Giza, Egypt*
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ABSTRACT

The elimination of adverse toxic effects of mycotoxins is currently the main strategy in animal production, particularly in poultry. The current study investigated the influence of chronic administration of deoxynivalenol on the health status, biochemical and immunological parameters of broiler chickens and the efficacy of ZnO-NPs and probiotics in preventing and treating the effect of toxicity. The experiment program lasted 6 weeks and was performed on a total of 60 broiler chickens aged 5 days, divided into six groups. Group 1 received healthy feed free of toxins, group 2 was fed with deoxynivalenol (DON), group 3 received Zinc Oxide nanoparticles (ZnO-NPs) and DON, group 4 had ZnO-NPs for 1 week, then DON was added for the remaining 5 weeks, group 5 was fed on ZnO-NPs, 1 g probiotic powder/kg of diet, and DON, group 6 had ZnO-NPs and 1 g probiotic powder/kg of diet for 1 week, then DON was added for 5 weeks. The used dose of ZnO-NPs was 50 ppm, and DON was 5 ppm in the diet. The intoxicated chickens showed adverse changes as increased pro-inflammatory cytokines, serum hepatic, and pancreatic enzymes, as well as decreased free amino acids. The supplementation of ZnO-NPs and/or probiotics improved all toxic changes resulting from DON toxicity, indicating that the metal nanoparticles and probiotics can be used together in poultry feed to avoid the addition of high doses of ZnO-NPs. Therefore, the use of 50 ppm of nanomaterial supplementation plus 1 g probiotic/ kg feed for the degradation of mycotoxins in poultry feed is recommended as it is safe and affordable.

Keywords: Deoxynivalenol, *Fusarium* spp., Nanoparticles, Poultry, Probiotic

INTRODUCTION

Worldwide morbidity and mortality from mycotoxicosis in humans and animals have a negative economic impact due to decreased health activities and output. Trichothecenes are secondary metabolites produced by *Fusarium* species, such as *F. oxysporum*, *F. poae*, and *F. graminearum* with specific references to trichothecene (T-2), deoxynivalenol (DON), and nivalenol (NIV, Chen et al., 2017; Springler et al., 2017).

Deoxynivalenol mycotoxin that has been discovered in food, may cause serious health problems and dangers to humans and animals. The toxicity occurs due to the conjugation of epoxide group with DNA subunits, which prevents protein synthesis and causes injury to human and animal tissues (Awad et al., 2013). Acute consumption of high doses of DON may cause several gastrointestinal diseases, such as emesis and diarrhea, while chronic consumption results in growth reduction and dangerous effects on digestive organs, as well as anemia, carcinogenesis, tremors, hemorrhage, dermatitis, pulmonary edema, immunosuppression, and hormonal imbalances in humans and animals (Rotter, 1996; Pestka, 2010). Poultry production is negatively affected by DON. The mentioned results are related to the dysfunction of the gastrointestinal tract, predisposition to infectious diseases

by the suppression of the cellular and humoral immune system through immune cell necrosis, serum immunoglobulins reduction, and spleen atrophy (Reddy et al., 2018; Riahi et al., 2020; Hassan et al., 2022).

Mycotoxins-management strategies have gained a lot of attention to preserve the health of animals and poultry. Although most conventional drugs are still used to treat these diseases, lower efficacy has been found due to the emergence of germ resistance, which requires finding innovative therapeutic medicines.

Nowadays, nanotechnology applications are of worldwide interest in all biomedical fields (Moghimi et al., 2005). Currently, zinc oxide nanoparticles (ZnO-NPs) have been shown to suppress microbial development, as well as enhancing appropriate antibacterial and antifungal action, and unique qualities, such as fewer environmental hazards and effectiveness in suppressing the growth of toxigenic fungi and their capacity to produce poisons (Reddy et al., 2007; Alghuthaymi et al., 2021). Moreover, immunity improvement, health promotion, and productivity in chickens can be seen as a result of ZnO-NPs (Lina et al., 2009). In addition, ZnO-NPs could couple with curcumin to diminish the viability of mycotoxin *Fusarium* spp. and inhibit the production of mycotoxins. A nanocomposite of ZnO-NPs and cinnamon oils may be able to remove *Fusarium* mycotoxins in animal feed (Gacem et al., 2021; Hassan et al., 2022).

Several studies have shown that probiotic formulations, particularly those containing *Lactobacillus* spp., have the potential to suppress fungal growth, and detoxify food and feed toxins (Fredua-Agyeman et al., 2017). The bio-detoxification of DON by microorganisms has been developed as a probiotic and has demonstrated significant toxin elimination benefits (Payros et al., 2016; Tiew et al., 2020; Chaudhari et al., 2022).

This study evaluated the toxicological effects of DON on broiler chickens and the efficacy of ZnO-NPs and/or probiotics in degrading the toxic effects of DON.

MATERIAL AND METHODS

Ethical approval

The experiment was approved by the Institutional Animal Health Research Ethics Committee, Egypt, and followed local laws and regulations (Vide Ref No. VMC/2014/IAEC/1046-73).

Feed samples

A total of 60 feed samples were collected from animal farms where chickens displayed toxicity symptoms, such as vomiting, diarrhea, anorexia, weight loss, and/or abrupt death. Every 500 g of feed sample was kept in a clean plastic bag and transported to the laboratory for analysis.

Zinc nanoparticles

The ZnO-NPs had a tiny particle of 50 nm in spherical forms that were prepared at the Nuclear Research Centre, Atomic Energy Authority, Egypt.

Probiotic vials

Micro-Procell vials each included 1 g of powder containing strains of *Lactobacillus plantarum* ($1x10^8$ CFU), and *Lactobacillus acidophilus* ($1x10^8$ CFU) were provided by Cheil Bio Co., Ltd, South Korea. *Saccharomyces cerevisiae* ($1x10^7$ CFU) with 0.5 g skim milk carrier was provided by Lallemand, SAS, France, under the name Levucell SB 10ME Titan (LSB).

Deoxynivalenol standard solution for mycotoxin detection using Thin Layer Chromatography

Mycotoxin's standard of DON was purchased from ALDRIK Sigma Chemical Company (St. Louis, USA).

Isolation and identification of *Fusarium* in feed samples

Under the aseptic process, 1 g of each feed sample was placed into sterile tubes containing 9 ml sterile distilled water to obtain tenfold serial dilution. Each ml of the serial dilutions was individually put into sterile petri dish plates and mixed with Sabouraud's dextrose agar containing 0.05 mg of chloramphenicol and incubated aerobically at 25°C for 3-5 days according to ISO (2008). *Fusarium* species were identified by macroscopic and microscopic features of mold colonies by Pitt and Hocking (2009).

Production of deoxynivalenol mycotoxin

The cultures of mycotoxigenic *Fusarium graminearum* isolates were cultivated on Potato Dextrose Agar (PDA) for 5-7 days at 25ºC. A total of 500 ml flasks with 100 gm of fine grounded yellow corn were autoclaved for 1 hour at 121ºC. Then, slant spores of each *Fusarium* species of 1 week old were added to the autoclaved flask and incubated for 4 weeks at 25-28ºC. The flasks were transferred to the refrigerator at 8-10ºC for 2 more weeks to produce a toxin (D'Mello et al., 1998). The produced trichothecenes mycotoxins were extracted and measured by thin-layer chromatography according to

studies by Kamimura et al. (1981) and Bottalico et al. (1985).

Chickens

A total of 60 broiler chickens aged 5 days from Dyerna farm, Giza, Egypt, were kept in clean cages while healthy feed and water *ad libitum* were available for 6 weeks. At 7 days of age, chickens were vaccinated against Newcastle and infectious bronchitis diseases using the HIPRAVAR Hitchner B1+H120 vaccine (HIPRA, Girona, Spain) via an intra-ocular route. At 10 days of age, the vaccination against avian influenza virus was done using CEVA C® NEW FLU - KEM inactivated H5N2 vaccines (CEVA, Giza, Egypt) via subcutaneous route.

Grouping

Chickens were divided into six groups. For the first group, healthy diet was available free of toxins, the second group received only DON and kept as intoxicated chickens, the third group was fed with ZnO-NPs and DON, the fourth group received ZnO-NPs for a week, then DON was added to the ZnO-NPs, the fifth group was fed on ZnO-NPs, 1 gm probiotic powder/kg of diet, and DON, finally, the sixth group received ZnO-NPs and 1 gm probiotic powder/kg of diet for a week, then DON was added. The utilized dosage of ZnO-NPs was 50 ppm and DON was 5 ppm in the diet. The experiment lasted 6 weeks following the studies of Yalcinkya et al. (2012) and Ahmadi et al. (2014).

Sampling

At the end of the experiment, 5 ml blood samples were collected from each chicken's brachial (wing) vein using a 20-gauge needle 1 inch in length. The serum was separated by centrifugation (SHengwin, USA) for 15 minutes and immediately frozen at -20ºC until analysis. According to AVMA Guidelines for the euthanasia of animals (Underwood and Anthony, 2020), Chickens were euthanized by cervical dislocation to collect livers and muscles from different groups to detect DON mycotoxin residues.

Serum biochemical parameters

Total protein was estimated according to Sonnen-Wirth and Jaret (1981). Assays for alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, and lipase levels in serum were determined according to Lopez (2013). Malondialdehyde (MDA) and Glutathione (GSH) were determined following Ellman (1959), and Okhawa et al. (1979), respectively. Superoxide dismutase (SOD) was estimated according to Nishikimi et al. (1972). Amino acid analysis using the Amino Acid Analyzer sykam GmbH, Analytischer Messtechnik (Gewerbering 15, D_86922 Erosing, Germany) in the Genetic Research Center, Giza, Egypt.

Cytokines detection

Interleukin 6 (IL6) and the tumor necrotic factor-α (TNF-α) were measured by Nori chicken IL6 ELISA kits and Nori chicken TNF alpha ELISA kits, respectively (Genorise scientific, Inc., Pennsylvania, USA) according to the manufacturer's protocol**.**

Measurement of deoxynivalenol residues

Liver and muscle tissues were subjected to direct examination using a fluorometric assay to determine Deoxynivalenol residues, according to Kongkapan et al. (2016).

Statistical analysis

The obtained data were analyzed in SPSS, version 16 using ANOVA F-test and presented as mean ± standard error. The estimation of a significant difference was set at $p < 0.05$ (SPSS, 2007).

RESULTS AND DISCUSSION

Probiotics improve the health and immunity of animals infected with mycotoxins through inhibiting their cellular permeability and preventing their carcinogenic effects by enhancing their biodegradation (Ghadaksaz et al., 2022).

As presented in Table 1, *F. graminearum* produced higher levels of DON, T-2, Diacetoxy-scirpenol (DAS), and NIV resulting in higher total trichothecenes, compared to *F. sporotrichioides*. These toxins produced by *F. sporotrichioides* and *F. graminearum* negatively affect immunity and inhibit protein synthesis (Black et al., 1992; Osweiler, 2000). The higher level of DON in poultry feeds was reported as 5 mg/kg according to European Commission (2006). However, the lower levels of DON sometimes resulted in adverse effects on the suppression of chicken health status (Atanasova-Penichon et al., 2012; Lucke et al., 2017). Moreover, the administration of DON mycotoxins within the range of 5-15 ppm in the chicken feed negatively influenced productive performance, metabolic markers, and spleen cell growth, leading to DNA damage (Chen et al., 2017; Riahi et al., 2020). In addition, DON mycotoxin affects lipid peroxidation and DNA damage in chicken lymphocytes (Awad et al., 2012;

Ghareeb et al., 2015). The health of the internal organs of poultry, as well as biochemical and immunological markers, are influenced by the levels of 1-5 ppm, which causes jejunal and other digestive organ anomalies in hens.

Table 2 demonstrates a decrease in antioxidant activity by DON (G2), as measured by the significant reduction of SOD activity and GSH levels compared with (G1) ($p < 0.05$), and an increase of oxidative stress, measured by MDA levels, compared with control group ($p < 0.05$). Meanwhile, the administration of ZnO-NPs and/or probiotics ameliorated the damage caused by DON as shown by the elevated SOD, GSH, and lowered MDA levels compared with (G2). Antioxidant enzymes are critical in the defense against xenobiotic-induced oxidative damage. Deoxynivalenol is a polar molecule with three free hydroxyl groups (-OH), which contributes to oxidative stress by forming a high number of free radicals with an excess of oxidation markers, such as lipid peroxidation, MDA, and depleting antioxidants enzymes, including SOD and catalase, resulting in membrane and DNA damage (Nagy et al., 2005; Miloradovic et al., 2008; Wu et al., 2014).

Dawei et al. (2009) and Hu et al. (2012) found the ability of ZnO-NPs to scavenge free radicals and suppress the incidence of cell damage and carcinogenesis. Furthermore, the administration of ZnO-NPs enhanced the effectiveness of digestion and hence the availability of body nutrients. Moreover, Gacem et al. (2021), Mandal (2021), and Singh et al. (2021) confirmed that ZnO-NPs significantly elevated the antioxidant activity of cell membrane enzymes and reduced MDA. Probiotic strains that can restrict excessive levels of reactive radicals *in vivo* may prevent and treat various disorders related to oxidative stress. Probiotics elevate antioxidant defenses by producing vitamins and releasing GSH that are absorbed and dispersed throughout the body (Spyropoulos et al., 2011; Kushkevych and Jampílek, 2021; Hassan et al., 2022).

Table 1. Prevalence of trichothecenes mycotoxins produced by the isolated *Fusarium* spp. from poultry feeds

Trichothecene mycotoxins (ppm) / *Fusarium* spp. (5 strains of each)	DON (Mean ± SE)	T-2, DAS, NIV (Mean ± SE)	Total trichothecene (Mean ±SE)
F. graminearum	22.5 ± 0.60	12.5 ± 1.5	35.0 ± 2.2
F. sporotrichioides	13.5 ± 1.9	7.5 ± 0.83	21 ± 1.95

SE: Standard error, *F*: *Fusarium*, DON: Deoxynivalenol, T-2: Trichothecene, DAS: Diacetoxy-scirpenol, NIV: Nivalenol.

Table 2. Effects of dietary supplementation of ZnO-NPs and/or probiotic on oxidative stress and antioxidant activities in broiler chickens contaminated with deoxynivalenol

Groups	MDA (nmol/ml)	GSH (u/ml)	SOD (u/ml)
G1	$1.09^{c} \pm 0.04$	$6.90^{a} \pm 0.06$	$55.52^{a} \pm 1.02$
G2	$1.70^{a} \pm 0.08$	$4.83^{c} \pm 0.13$	$28.00^{d} \pm 1.89$
G3	$1.38^{b} \pm 0.03$	$5.53^{b} \pm 0.13$	$30.33^{c} \pm 1.45$
G4	$1.63^{a} \pm 0.08$	$5.18^{bc} \pm 0.23$	$34.30^{c} \pm 1.93$
G5	$1.29^{bc} \pm 0.12$	$5.68^{b} \pm 0.18$	$38.33^{bc} \pm 2.73$
G6	$1.28^{bc} \pm 0.03$	$5.66^{b} \pm 0.17$	$44.33^{b} \pm 5.99$

Data are represented as mean value ± Standard error. Values in the same column with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for 1 week then DON + ZnO-NPs + probiotic. ZnO-NPs: Zinc oxide nanoparticles, MDA: malondialdehyde, GSH: Glutathione, SOD: superoxide dismutase.

Table 3. Effects of dietary supplementation of ZnO-NPs and/or probiotic on total protein and some enzyme activities in broiler chickens contaminated with deoxynivalenol

Groups	TP (g/dl)	ALT (U/l)	AST (U/l)	Amylase (U/l)	Lipase (U/l)
G1	$4.10^{a} \pm 0.07$	$29.00^{d} \pm 1.53$	$54.00^{d} \pm 0.58$	$881.67^{e} \pm 1.76$	$1.05^{c} \pm 0.03$
G2	$3.07^{c} \pm 0.12$	$54.67^{a} \pm 2.60$	$93.70^{a} \pm 1.82$	$1244.65^{a} \pm 5.53$	$1.25^{a} \pm 0.05$
G3	$3.58^{b} \pm 0.06$	$40.00^{b} \pm 0.58$	$84.33^{b} \pm 1.67$	$1002.67^{c} \pm 29.21$	$1.14^{bc} \pm 0.01$
G4	$3.53^{b} \pm 0.03$	$41.67^{b} \pm 0.33$	$82.63^{b} \pm 0.82$	$1115.67^{b} \pm 0.67$	$1.19^{ab} \pm 0.04$
G5	$3.71^{b} \pm 0.03$	$35.33^{c} \pm 1.20$	$72.37^{c} \pm 0.74$	$975.00^{cd} \pm 4.58$	$1.13^{bc} \pm 0.01$
G6	$3.72^{b} \pm 0.06$	$34.67^{c} \pm 1.45$	$74.67^{c} \pm 2.03$	$936.32^{d} \pm 18.18$	$1.06^{c} \pm 0.01$

Data are represented as mean value ± Standard error. Values in the same column with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for 1 week then DON + ZnO-NPs + probiotic. ZnO-NPs: Zinc oxide nanoparticles, TP: Total protein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Table 3 presents the total serum protein (TP), ALT, and AST as indicators of chicken metabolism and visceral organs' health. According to Pestka, (2010), DON is a powerful protein synthesis inhibitor, causing protein turnover disruption even at low concentrations. Compared to the control group, serum ALT and AST levels have also been found to be sensitive markers of liver damage since a rise in these values in the DON treatment groups indicates leakage of injured hepatocytes following DON intake (Nyblom et al., 2004).

The pancreatic enzyme showed a significant rise in serum amylase and lipase activity after DON intake ($p < 0.05$). Similarly, according to previous studies, DON enhanced the activity of pancreatic chymotrypsin, amylase, and lipase (Richardson and Hamilton, 1987; Matur et al., 2010). On the contrary, Osborne et al. (1982) believe that DON ingestion causes malabsorption, defined as steatorrhea, hypocarotenoidemy, and reduces bile, pancreatic lipase, trypsin, and amylase. The administration of ZnO-NPs to the contaminated groups resulted in lower levels of liver function indicators, such as ALT and AST, compared to the non-treated group (Table 3). These findings agree with Ahmadi et al. (2014), who demonstrated that dietary ZnO-NPs decreased serum ALT in broiler chickens. ZnO-NPs also significantly reduced amylase and lipase levels ($p < 0.05$), resulting in a lower histopathological damage score in both the pancreas and the liver tissues.

Table 4 presents that the mycotoxicated group (G2) had a significant elevation of the pro-inflammatory cytokines, including both interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) compared with G1 ($p < 0.05$). Cytokines produced by immune cells have significant functions in immune response (Giansanti et al., 2006). Similar findings to the current results were reported by Awad et al. (2013) and Hendrayani et al. (2016), who found that the administration of deoxynivalenol contaminated diet in chickens increased serum levels of IL-6 and TNF-α. Another study indicated that exposure to DON potentiated inflammatory gene expression and the chronic doses resulted in necrosis and death cells (Shi et al., 2009; Wu et al., 2015; Peng et al., 2019). Furthermore, ZnO-NPs and/or probiotics treatment in groups 3, 4, 5, and 6 showed anti-inflammatory activity by IL-6, and TNF-α. The results are consistent with earlier research by El-Bahr et al. (2020), Mandal (2021), and Sakthivel et al. (2021), who found that ZnO-NPs increased the levels of antioxidant enzymes, including SOD, catalase, and glutathione peroxidase and decreased IL-6 and TNF-α cytokines in liver and brain tissues. The current study revealed lower levels of free amino acids in G2 (Table 5). After DON exposure, the observed initial adverse effects included reduced feed intake, emesis, diarrhea, and anorexia (Pestka and Smolinski, 2005). Moreover, DON exposure negatively affected the absorption and metabolism of different biological and intestinal flora resulting in adverse toxic effects (Awad and Zentek, 2015). It was suggested by Wu et al. (2014) that reduced levels of amino acid in contaminated chicks with DON are due to intestinal barrier dysfunction. Table 5 shows that contaminated chickens with DON (G2) adversely affected the health status of chickens by reducing levels of amino acids. However, the supplementation of ZnO-NPs and/or probiotics improved amino acids levels in groups 3, 4, 5, and 6. The amino acids are essential for protein synthesis, and reduced absorption because of DON toxicity resulted in losses in the productivity of poultry (Wu et al., 2015).

The administration of ZnO-NPs in group 3 manifested significant increase in serum amino acid of chickens, compared to the second group ($p < 0.05$). According to Zhang et al. (2018), leucine, isoleucine, and proline were elevated by ZnO-NPs, compared to the normal control. The serum lysine content in the ZnO-NPs and/or probiotic groups increased in comparison with the second contaminated group due to increase amino acid absorption in treated chickens (Wu et al., 2015). The present study detected that the probiotic administration in contaminated chicken feed helped the chicken overcome the toxic effects of DON on some vital organs, such as liver, this agreed with (Marzouk et al., 2008; Mehrim, 2009). Recently, it has been reported that the probiotic preparation initiated the immune function of phagocytosis and epithelial cells against DON toxicity and microbial infection (Vinderola and Ritieni, 2015; Chaudhari et al., 2022; Hassan et al., 2022). Moreover, the elimination of DON by the supplementation of probiotic strains in the feed may be due to the occurrence of malabsorption of toxins in the gastrointestinal tract of treated livestock and reduced oxidative stress and inflammatory response (Chen et al., 2013; Zhao et al., 2013; Vila-Donat et al., 2018).

Previous studies evaluated the elimination of *Fusarium* toxins (Trichothecenes; DON) from food using *bacillus* probiotics (Zhao et al., 2016; Wang et al., 2020; Thipe et al., 2022).

According to Table 6, the second group received only DON and had the highest levels of DON in liver and muscle tissues. The third group received ZnO-NPs with DON and had better elimination of DON in the liver and muscles. The trials of administration of ZnO-NPs and or probiotics in the fourth and sixth groups before the addition of DON showed lower levels of detoxification. However, the residues of DON in the liver after treatment were still within the permissible limits, as reported by Escrivá et al. (2017).

Table 4. Effects of dietary supplementation of ZnO-NPs and/or probiotic IL- 6 and TNF- α: of broiler chickens contaminated with deoxynivalenol

Parameters	G1	G2	G3	G4	G5	G6
IL- 6 (ng/l)	$121.51^{d} \pm 2.19$	$147.83^{a} \pm 3.63$	$130.33^{bc} \pm 3.38$	$137.33^{b} \pm 1.67$	$128.33^{c} \pm 1.45$	$135.00^{bc} \pm 2.31$
TNF- α (ng/l)	$69.67^{d} \pm 2.03$	$112.33^{a} \pm 6.94$	$98.00^{ab} \pm 6.51$	$109.67^{a} \pm 4.84$	$87.67^{b} \pm 4.70$	$104.67^{ab} \pm 5.17$

Data are represented as mean value ± Standard error. Values in the same row with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for one week then DON + ZnO-NPs + probiotic. ZnO-NPs: Zinc oxide nanoparticles. IL- 6: Interleukin-6, TNF- α: Tumor necrosis factor-α

Table 5. Effects of dietary supplementation of ZnO-NPs and/or probiotic on some serum amino acids (g/dl) of broiler chickens contaminated with deoxynivalenol

Groups	Lysine	Methionine	Leucine	Iso-leucine	Arginine	Proline
G1	$2.09^{a} \pm 0.01$	$1.33^{a} \pm 0.03$	$2.43^{a} \pm 0.05$	$1.72^{a} \pm 0.03$	$2.62^{a} \pm 0.03$	$1.00^{a} \pm 0.01$
G2	$1.61^{c} \pm 0.05$	$1.11^{c} \pm 0.01$	$2.05^{b} \pm 0.03$	$1.21^{d} \pm 0.02$	$2.14^{cd} \pm 0.04$	$0.65^{d} \pm 0.04$
G3	$1.79^{b} \pm 0.03$	$1.26^{ab} \pm 0.05$	$2.18^{b} \pm 0.02$	$1.33^{c} \pm 0.00$	$2.21^{c} \pm 0.01$	$0.90^{b} \pm 0.01$
G4	$1.70^{bc} \pm 0.02$	$1.13^{c} \pm 0.02$	$2.09^{b} \pm 0.01$	$1.31^{c} \pm 0.01$	$2.30^{bc} \pm 0.04$	$0.80^{c} \pm 0.03$
G5	$1.74^{b} \pm 0.06$	$1.24^{b} \pm 0.01$	$2.16^{b} \pm 0.03$	$1.37^{c} \pm 0.03$	$2.38^{b} \pm 0.03$	$0.91^{b} \pm 0.01$
G6	$1.79^{b} \pm 0.03$	$1.22^{b} \pm 0.02$	$2.15^{b} \pm 0.12$	$1.54^{b} \pm 0.06$	$2.30^{bc} \pm 0.04$	$0.95^{ab} \pm 0.02$

Data are represented as mean value ± Standard error. Values in the same column with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for one week then DON + ZnO-NPs + probiotic, ZnO-NPs: Zinc oxide nanoparticles

Table 6. Levels of deoxynivalenol residues in the liver and muscles (ppb) of contaminated broiler chickens

Organs	G1	G2	G3	G4	G5	G6
Liver	ND	1.56 ± 0.14	0.51 ± 0.20	0.31 ± 0.15	1.1 ± 0.27	1.3 ± 0.20
Muscles	ND	1.6 ± 0.25	0.05 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.03 ± 0.00

G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for one week then DON + ZnO-NPs + probiotic DON. ZnO-NPs: Zinc oxide nanoparticles, ND: Not detectable

CONCLUSION

According to the findings, *Fusarium* species isolated from chicken feeds produced significant amounts of Trichothecenes mycotoxins, particularly DON. In the present investigation, this toxin has a detrimental effect on the health status, immunological state, and output of chickens. Furthermore, supplementation of ZnO-NPs, either alone or in combination with the probiotic, showed considerable potential in eliminating the negative activity of DON in broiler chickens. As a result, supplementation of ZnO-NPs and probiotic can significantly improve poultry and animal health, as well as their products such as meat, eggs, milk, wool, and leather. Finally, it is recommended to conduct future studies on the addition of probiotics to ZnO-NPs for the degradation of mycotoxins and lowering the used doses of nanomaterials to avoid their toxic levels.

DECLARATIONS

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Authors' contribution

Rasha Sayed-ElAhl designed the research. Rasha Sayed-ElAhl, Atef Hassan, Mogda Mansour, Azza Abdelmoteleb, and Ahmed El Hamaky performed the experimental duties of this study and analyzed the data. Mogda Mansour did the statistical analyses. Atef Hassan and Mogda Mansour wrote the draft of the manuscript.

Mogda Mansour, Rasha Sayed-ElAhl, Azza Abdelmoteleb, and Ahmed El Hamaky have taken part in the revision of the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors investigated ethical issues such as plagiarism, permission to publish, malfeasance, data falsification and/or fabrication, double publishing and/or submission, and redundancies.

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A Microscopic Study on Morphology of Reactive Thrombocytes in Duckling

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ABSTRACT

Thrombocytes, well known as important clotting factors, and now known to be important as phagocytic cells, might benefit the study of the avian hemogram. Therefore, blood was sampled from 4 late-stage embryos at embryo day 24, and 4 one-day-old hatchlings (d1), and 5 female ducks aged 59 weeks stained by Wright-Giemsa and examined at 100x. Standard differential counts (SDC) of 2 x 200 cells were used to determine total white blood counts (TWBC) and heterophil/lymphocyte (H/L) ratios**.** Thrombocytes were not included in the SDC but were studied from photomicrographs. Reactive thrombocytes were present in blood films having a normal TWBC or in the presence of leukocytosis (59 weeks). The H/L ratios may or may not be elevated. Reactive thrombocytes can be differentiated from quiescent types on morphologic criteria. These included an increase in the number of magenta "specific granules", the development of cytoplasmic vacuoles, and a capacity to form aggregates with other Th or with cells of another series. Reactive Th were not necessarily larger in size than quiescent types. In some instances, Th aggregation with RBC (toroid formation) was with sufficient force to distort the RBC cell membrane. It was observed that reactive thrombocytes were accompanied by bacteria, either free-swimming or attached to cell-associated bacteria. Reactive thrombocytes having lost portions of their cell membrane were regularly encountered. As avian thrombocytes are now recognized as important phagocytic cells, as well as having a primary role in hemostasis, they are part of the immune defense mechanism. The presence of reactive thrombocytes in a hemogram should be considered when using hematological data to evaluate immune responses and establish stress status.

Keywords: Hematology, Immunity, Reactive thrombocyte, Simple and complex toroid, Stress

INTRODUCTION

Avian thrombocytes (Th) appear to parallel the prominent role in hemostasis played by mammalian platelets (Ferdous and Scott, 2015). They initiate clotting, repair damaged endothelium, and accumulate at the site of injury where they block blood leakage (King, 1980). One study suggested they are less important in (early) clotting due to a deficiency of thromboplastin (Handlinger, 1989). Morphological changes in thrombocytes associated with stress were described by Gross (1989) who classified thrombocytes with a system of scores.

In normal circulation, thrombocytes are more numerous than other leukocytes occurring at approximately 30K/µL in chickens (Lucas and Jamroz, 1961). Thrombocyte phagocytosis was demonstrated microscopically using a Trypan Blue dye particle uptake assay (Carlson et al., 1968). *In vitro* studies demonstrated duck thrombocytes change from oval shapes to spheres after exposure to adenosine diphosphate (ADP), and they are aggregation by shaking (Grant et al., 1973). Thus, a change of shape can also be a normal physiological response of thrombocytes and may account for some of their morphological variations (Lucas and Jamroz, 1961).

The phagocytic capacity of thrombocytes for several types of bacteria was investigated by Wigley et al. (1999) who showed activity against *Salmonella* and other Gram-negative species. Ferdous et al. (2008) indicated Interleukin-12 (IL-12) is produced by thrombocytes exposed to lipopolysaccharide (LPS). Toll-like receptor production by reactive thrombocytes accompanies the release of cytokines from their storage granules, suggesting thrombocytes respond to viruses as well as bacteria (St Paul et al., 2012). Collectively, these observations indicate the dynamic nature of the

thrombocytes. They participate in a variety of immune functions beyond their hemostatic duties. A recent review of avian thrombocyte biology, including observations on duck thrombocytes, outlining their morphological and immunological properties is reported by Astill et al. (2022). With this in mind, the purpose of the current study was to illustrate various cytological properties of thrombocytes as they transition from a quiescent to a reactive state.

MATERIALS AND METHODS

These observations came from blood sampled from commercial stocks not otherwise manipulated; or from older breeding stock ducks.

Late-stage embryos and day of hatch samples

The data were obtained from light microscopic observations of thrombocyte behavior in duck peripheral blood. Shape change, specific granule elaboration, vacuole development, phagocytosis, and adherence to other thrombocytes and leukocytes were illustrated. All were observed in late-stage embryos at embryo day 24 (Ed24), hatchlings at day 1 (d1), and 5 breeding ducks aged 59 weeks (59 weeks). Thrombocytes were often found in the presence of either free-swimming or cell-associated bacteria (CAB). Eight blood smears prepared at a hatchery were sent to Cotter Laboratory, Arlington, MA, USA, for the current study. The samples of jugular vein blood came from 4 Ed 24 and 4 d1 hatchlings. They were post-fixed by immersion in 95% EtOH for 15 minutes followed by staining (6 minutes) using an of Wright-Giemsa method (Cotter, 2021b).

Breeding ducks

Blood samples taken at 59 weeks came from a Berne, Indiana, USA flock chosen because of a history of poor fertility. Whole blood (1-3 mL) drawn from a leg vein was placed into EDTA tubes. To avoid the storage effects, monolayer films were made within 24 hours of collection. Approximately, 3 µL of blood was spread across the length of alcohol-cleaned glass microscope slides and dried immediately with a hot air stream. Slides were immersed in 95% ethanol and postfixed for 10-15 minutes. Staining was done as described above.

Welfare

Ducking welfare was monitored under the Maple Leaf Farms Trident Stewardship Program for Duck Well Being with procedures reviewed by a PAACO-certified auditor and licensed Veterinarian.

Light microscopy and photomicrographs

An Olympus CX-41 (Olympus America, Center Valley, PA 18034-0610) was equipped with Plan N 40x, 0.65 numerical aperture dry, and Plan N, 1.25 numerical aperture 100x oil objectives. Images were captured at 100x with an infinity-2, 1.4-megapixel charge-coupled device Universal Serial Bus 2.0 Camera, and processed with infinity analyze software (Release 6.5, Lumenera, Inc., Ottawa, ON, Canada).

RESULTS

The first illustrations of normal (quiescent) thrombocytes were observed in Ed24 and d1. These were followed by examples of cells illustrating changes in cytology from the normal (quiescent) to reactive states. Lastly, examples of reactive thrombocytes (toroids) in 59 weeks ducks were given. The standard differential counts (SDC) for Ed24 and d1 blood films are presented in Table 1. Detailed SDC data for the older ducks were not included. However, the average total white blood counts (TWBC) in 59 weeks flock was 42K with heterophil/lymphocyte$_1$ (H/L1) ~7 and heterophil/lymphocyte$_2$ (H/L2) ~1.8. These values indicate leukocytosis and stress, respectively.

Normal thrombocytes

Thrombocytes not displaying reactivity signs are classified as normal or quiescent cells. They may touch or overlap other cells with which they are not otherwise engaged. (Figure 1A, hatchling at d1). They are oval-shaped and contain 1 or 2 specific granules (magenta) usually located at opposite poles. Cell area (Ac) ~ 20 µm^2; Nuclear/Cytoplasmic Ratio (N/C) ~ 0.4. Generally, they occur as individuals within a field where they may overlap RBCs. Their pellucid cytoplasm appears devoid of hemoglobin. Figure 1B is a quiescent cell (Th1) in a field with CAB and free (encapsulated) bacteria located by asterixis at Ed24. The Th2 share a CAB with an RBC. A lymphocyte (Lp) is a large plasmacytoid cell displaying a paranuclear Hof (Golgi; Cotter, 2022). These examples appear in fields also containing an atypical classic heterophil (HC) and a reactive basophil (Cotter, 2017; Cotter, 2021).

Reactive thrombocytes

During the transition to the reactive state vacuoles develop in the thrombocyte cytoplasm (Figure 2A). The shape can change from an elliptical to a rounded form. This may occur without a noticeable increase in cell area (A_C ~20 μm^2) as illustrated by the paired cells in the lower corner (Figure 2B). The shape change is accompanied by the development of one or two ectactic vacuoles, here occupying only one pole. Vacuole development is sometimes accompanied by an increase in the number of specific granules. The former pellucid cytoplasm also acquires a slightly deeper stain. Surface changes detected microscopically are Th/Th adhesions and attachments to cells of another series. The overall picture of the transition from a normal quiescent field to reactivity is the replacement of space occupied by RBCs or thrombocytes with white blood cells (WBCs). The size of the nucleus in a nearby pRBC suggests it is a tetraploid (4C) cell containing twice the DNA of a diploid (2C) cell. The second example of a thrombocyte with an ectactic vacuole is at the right (Ed24 sample). A nearby RBC has an encapsulated cell associated bacteria (CAB) at its surface, and two non-attached bacteria are at the bottom center.

Adhesion of a thrombocyte to an eosinophil (Th/Eo) is shown in (Figure 3A) where the magenta-specific granules found at each pole appear to be separated from the cytoplasm by a clear space. An encapsulated bacterium has attached to the surface of a normal thrombocytes; at the left. Additional free bacteria located with asterixis and CAB are distributed throughout the field (Figure 3B). An example of phagocytic (located by an arrow) Th/Th attachment in a field where thrombocytes overlap RBC and free bacteria asterixis. The HC is a faintly stained (atypical) classic heterophil containing an intact phagocytosed bacterium. The presence of a capsule and attachment of bacteria to thrombocytes is not accidental but indicates a sub-microscopic change of the thrombocytes cm.

Thrombocyte deterioration was characterized by the loss of cytoplasm and specific granules as illustrated by the appearance of irregular-shaped cells (Figure 4B, Top). An absence of thrombocyte vacuoles was evident. The anuclear remnants of a net-type basophil that have entrapped multiple bacteria are located on the left (Cotter, 2017).

Variation of thrombocytes at Ed24 (Figure 5A). Th1 was normal and intact and has a full complement of cytoplasm, several specific granules, and no vacuole. Th2 was a reactive cell of the pseudopod type, possible equivalents of score 4 and 5 types described by Gross (1989). Th3 was a reactive cell nearly devoid of cytoplasm, N1 and N2 are nuclear remnants of uncertain origin. Background RBCs are normal and fully hemoglobinized but show some degree of overlapping (rouleaux). The variety of reactive and normal thrombocytes in a single field suggests the transition to a reactive state is an individual decision made by each Th, and perhaps under a stimulus initiated at a site remote from the photographic field.

A reactive thrombocyte was in a field in a hatchling (d1) along with an atypical heterophil (HC) and eosinophil (Eo) a reactive plasmacytoid lymphocyte (Lp); CAB (arrow) and free bacteria (circle). Figure 6B. Aggregated thrombocytes in an Ed24 field with free bacteria (asterixis) and CAB (arrows) and a polyploid blast cell (Bst).

The capacity of a thrombocyte to aggregate with another thrombocyte or a cell from another series, called a "toroid". It is illustrated in Figure 7A by a toroid composed of Th-RBC-Th aggregates found in a 59 weeks duck. One member of the toroid called a thromboplastid (Thp) is the anuclear equivalent of an erythroplastid, an anuclear RBC, often seen in duckling bacteremia. It contains an apparently intact phagocytosed bacterium (arrow in figure). Figure 7B is a toroid composed of multiple layers, abbreviated as $(Th\text{-}RBC\text{-}Th)^N$ anchored by a solitary bacterium at its center. It was found in the same duck. Toroid formation can cause a sufficient amount of intercellular force to result in distortions of the RBC cm as is seen here.

A scatter plot of H/L_2 versus H/L_1 for embryos, ducklings, and older ducks is in Figure 8. Cut-off values are those typically used to separate stress/non-stress hemograms for older avian samples. Neither H/L ratios nor the SDC appears remarkable (Table 1). However, as some of the data are from embryo and hatchling blood samples, it is recognized that the 0.4/0.5 H/L cut-off values are tentative.

Table 1. Standard differential counts in percentage based on four blood films each from embryonic day 24 and day 1 commercial duckling stock (2 × 200 cells/count).

Percentage	HT	HV	HC	Ls	Lm	NK	Bst	Mn	Ba	Eo	RT	Total	TWBC (K)	H/L 1	H/L 2	Δ H/L
Ed24	16.3	0.0	13.5	44.4	10.0	0.0	2.0	0.2	12.8	0.7	0.4	100	7.3	0.7	0.6	0.1
d1	33.3	0.0	32.1	24.3	5.3	0.0	0.4	0.0	3.9	0.7	0.4	100	45.6	2.8	2.4	0.5

H: Heterophil (HT typical, HV variant, HC classic) Ls: Small lymphocyte ~6µm diameter, Lm: Medium and large lymphocyte (diameter 8 - 10 µm), NK: Natural killer, Mn: Monocyte, Ba: Basophil, bst: Blast cell, RT: Reactive thrombocyte, Eo: Eosinophil. H/L 1 = (HT+HV+HC)/Ls; H/L 2 = (HT+HV+HC)/(Ls + Lm), ΔH/L = H/L 1- H/L 2; TWBC total white blood cells per cubic µL in thousands (K)

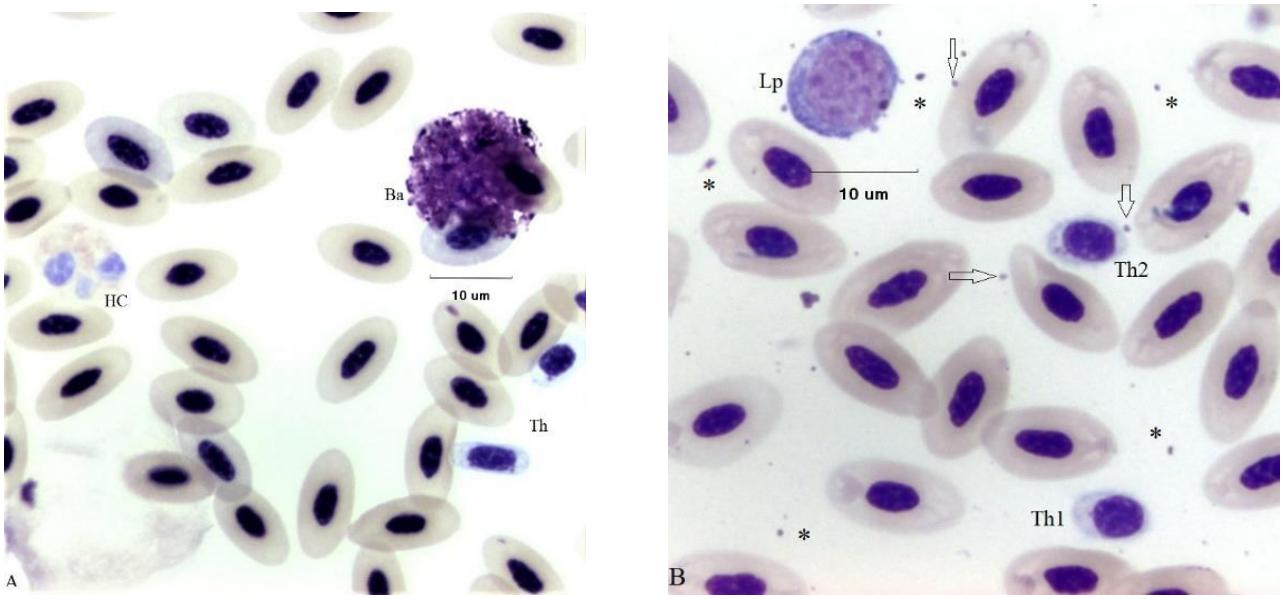

Figure 1. Normal thrombocytes (**A**). A pair of normal (quiescent) Th in a field with mature RBC, duckling at 1d. Each has 1 or 2 magenta specific granules, with A_C ~ 20 µm^2. An atypical heterophil (HC) and a reactive basophil (Ba) are nearby. Thrombocyte in a field also with bacteria (**B**).

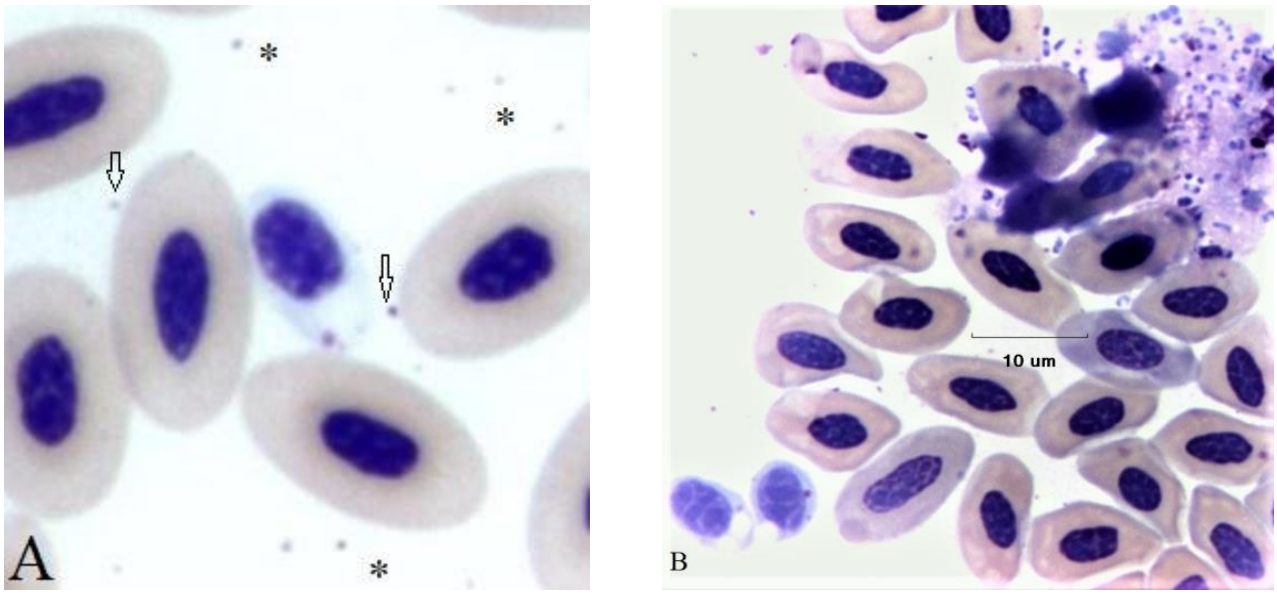

Figure 2. Reactive thrombocytes (**A**). Reactive thrombocyte with a conspicuous cytoplasmic vacuole, in a field with CAB (arrows) and free encapsulated bacteria (*). Reactive thrombocytes with cytoplasmic vacuoles and Th-Th attachment in a field with a polymicrobial colony (**B**); duckling d1.

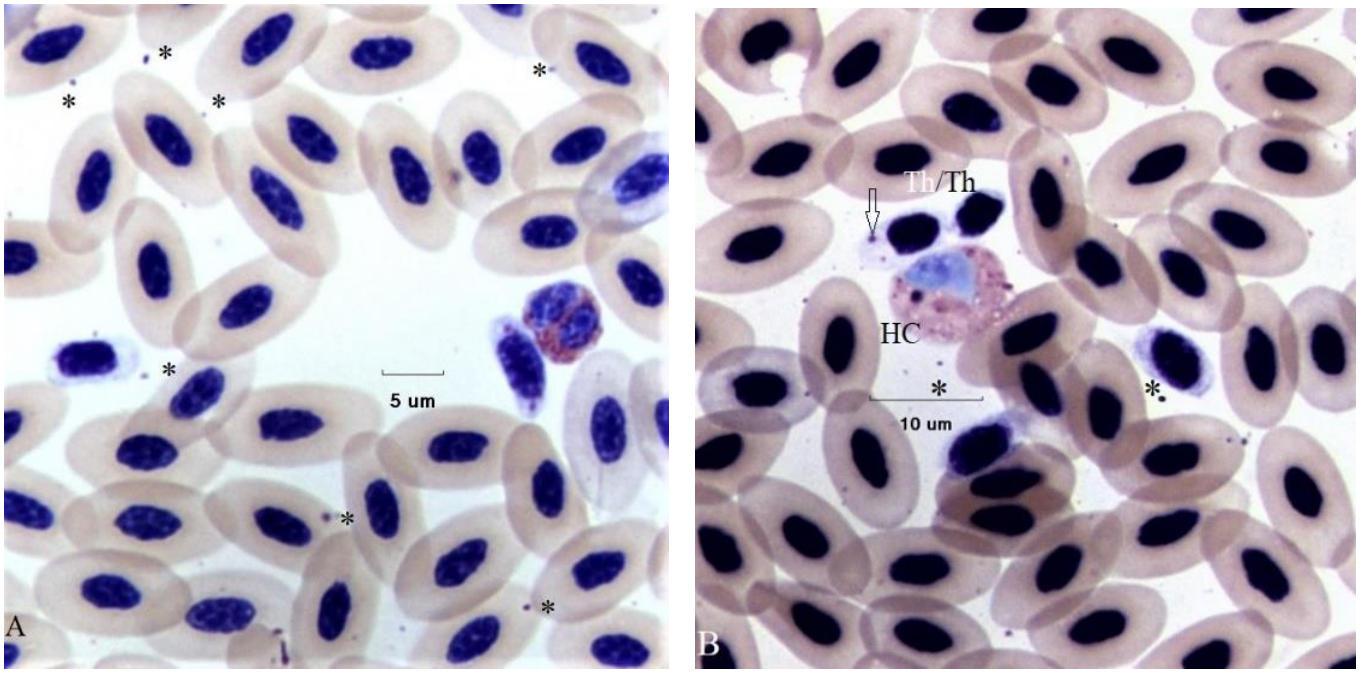

Figure 3. Thrombocyte attachments (**A**). The adhesive phase of thrombocyte reactivity can result in attachment to another thrombocyte or a cell of another series; a small eosinophil (Th/Eo). CAB (*) are also present (Ed24). An example of phagocytic (arrow) Th/Th attachment in a field with thrombocyte overlapping RBC and free bacteria (*). HC is an atypical classic (phagocytic) heterophil (**B**).

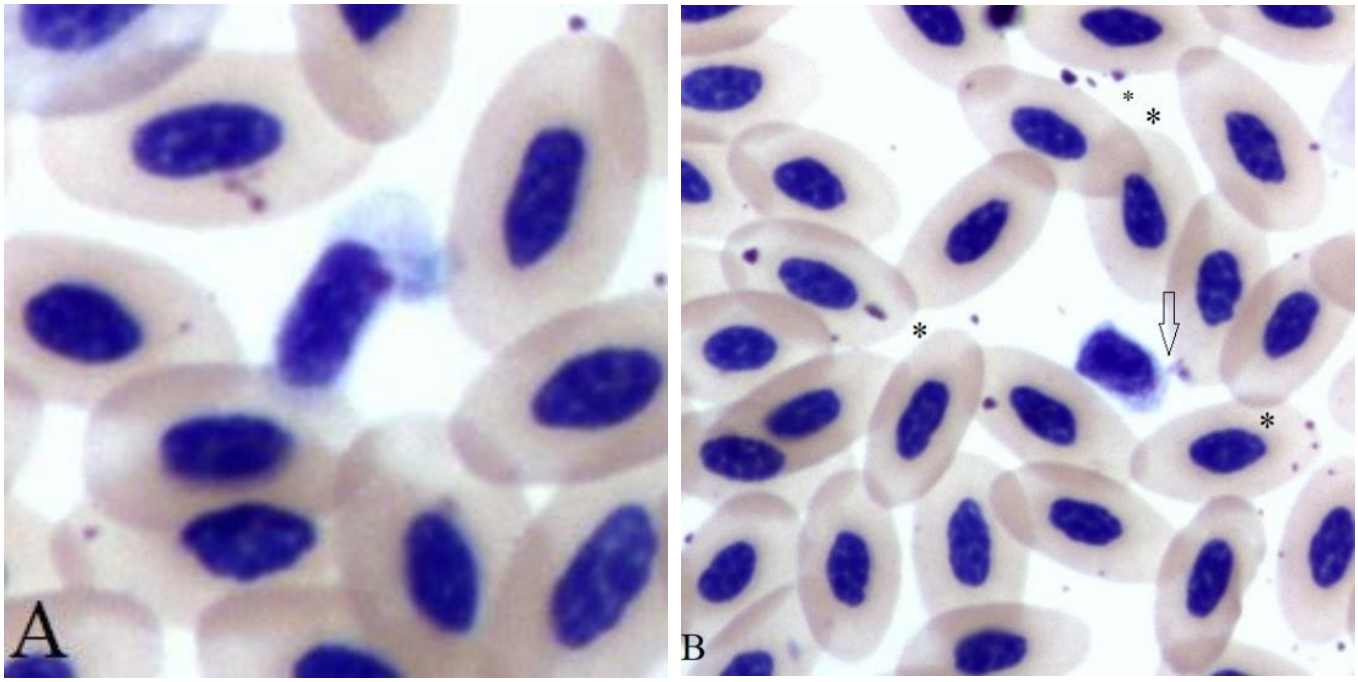

Figure 4. Cytoplasmic loss (**A**). Examples of reactive thrombocyte with partial (panel A) and more advanced loss of cytoplasm (panel **B**) found in the same sample (d1 duckling). CAB (arrow) and free bacteria (*) are also evident.

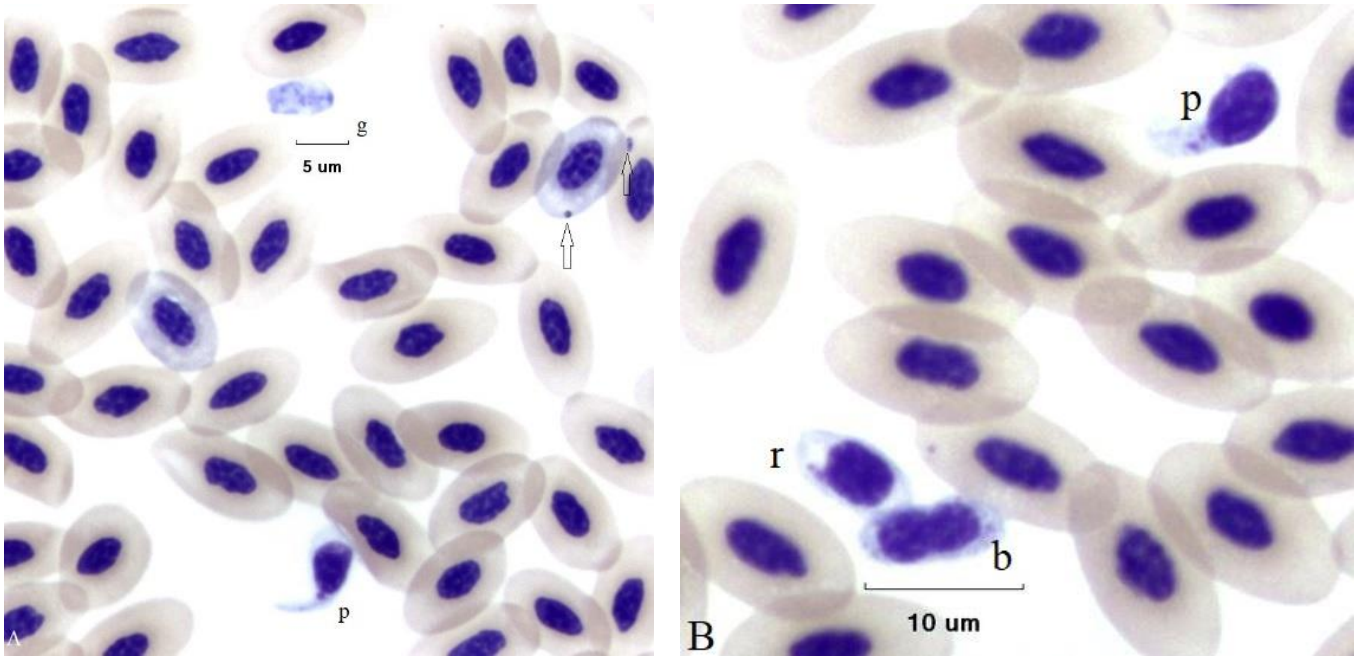

Figure 5. Pseudopod types (**A**). An example of a pseudopod type reactive thrombocyte in CAB fields. Multiple reactive thrombocyte in a single field: pseudopod (p) vacuolate (v) and (b) binuclear (Ed24, **B**).

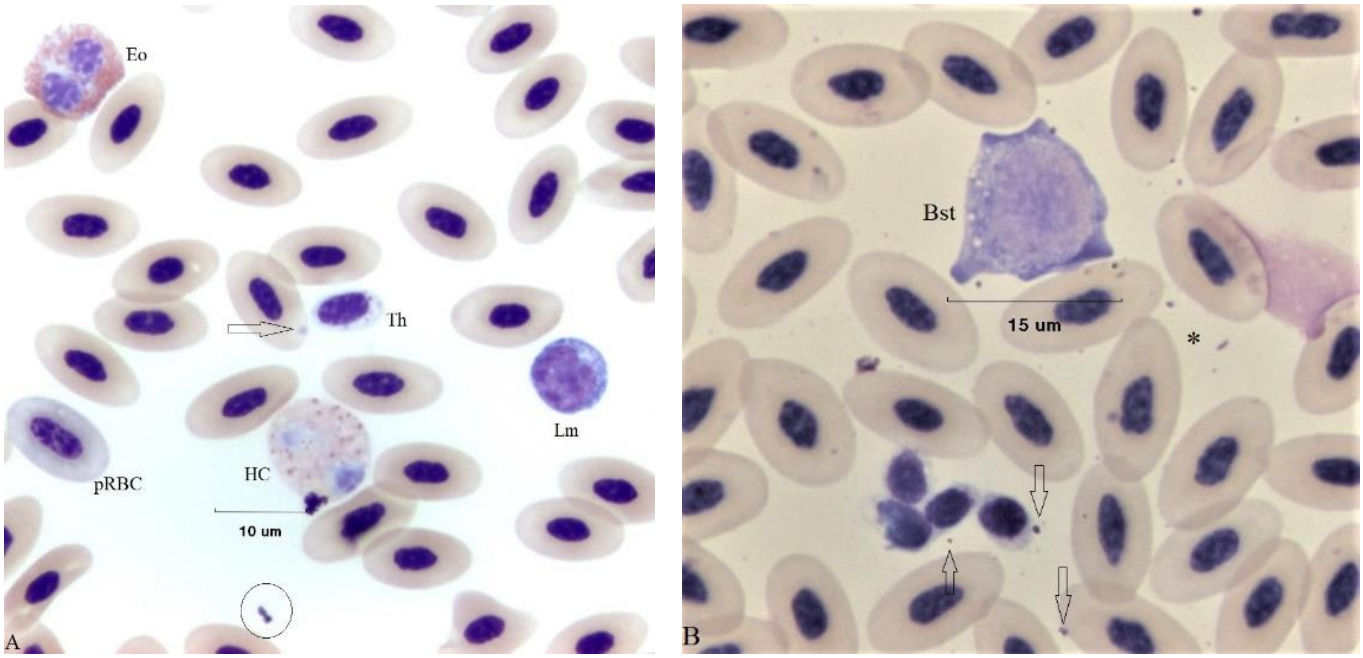

Figure 6. Thrombocytes accompanied by atypia of another series (**A**). Reactive thrombocyte in a field in a hatchling (d1) with an atypical heterophil (HC) an eosinophil (Eo) a reactive plasmacytoid lymphocyte (Lm) CAB (arrow) free bacteria (circle). Aggregated thrombocyte in a (Ed24) field with free (*) and CAB (arrows) and a polyploid blast cell (Bst, **B**).

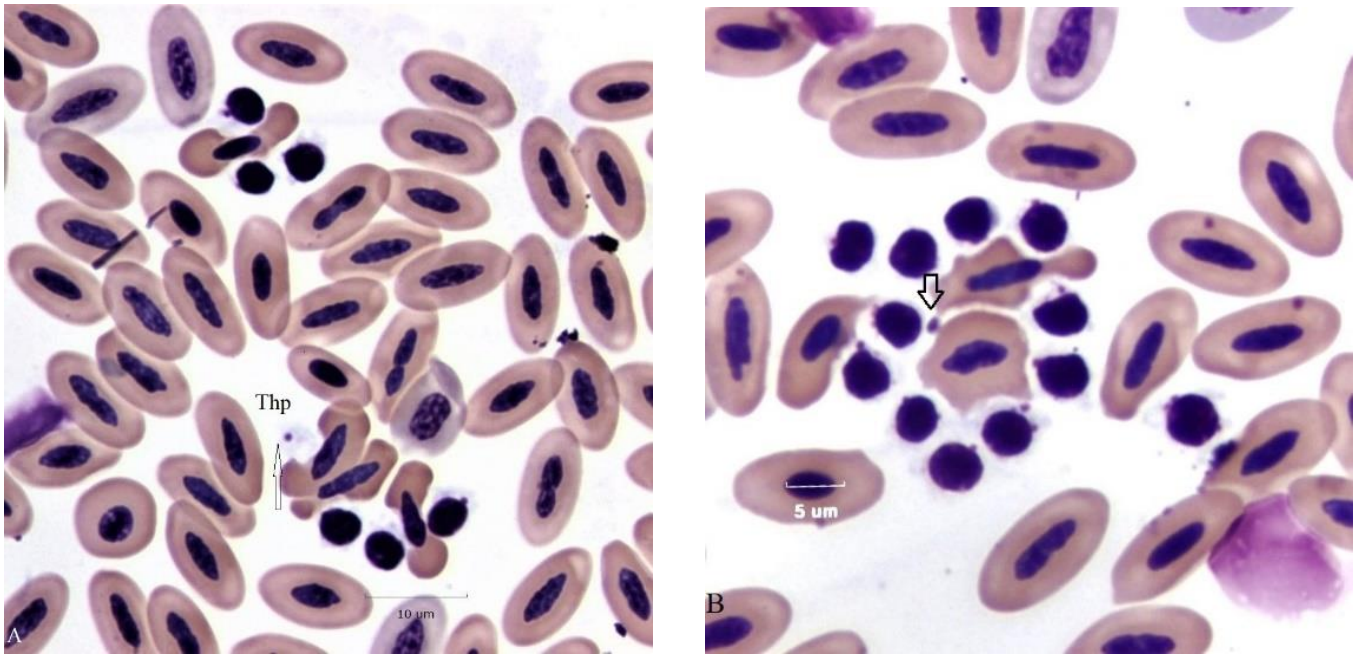

Figure 7. Toroid formation (**A**). Th-RBC toroid a phagocytic thromboplastid (Thp) containing an encapsulated bacterium (arrow). A top, a simple toroid, a more complex type. A multilayered toroid (Th-RBC-Th)^{N} in the same breeding duck at 59 weeks. A bacterium is located at the toroid center (arrow, **B**).

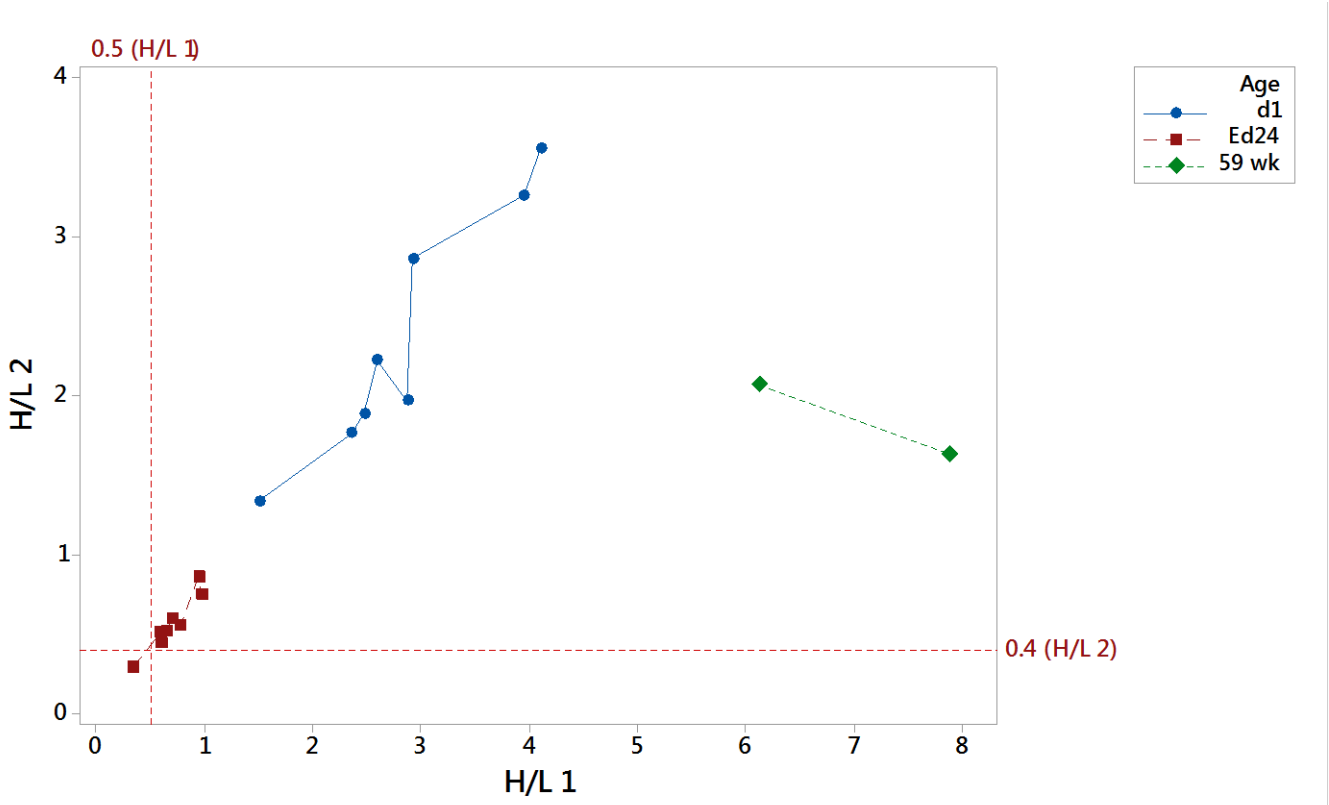

Figure 8. Scatter plot with stress/non-stress cut-offs of duplicate H/L 2 (0.4) vs. H/L 1 (0.5) of duckling blood computed from 2 x 200 cell SDCs sampled at Ed24 and d1. Wk 59 values are means (N=5).

DISCUSSION

The present observations indicated that the transition of quiescent thrombocytes to the reactive state was accompanied by a series of morphological changes some of which were recognizable at the level of ordinary light microscopy. Factors causing these changes are likely related to the presence of bacteria and their molecular products. These are assumed to influence the hemogram independently from factors influencing the H/L ratio. These included the development of vacuoles, an increase of specific granules, and the development of a capacity for adhesion to other thrombocytes and cells of another series. Some of these changes paralleled those described by Gross (1989) who studied crystal violet-stained thrombocytes at a lower magnification with an hemacytometer. Based on the present observations and additional studies (Cotter, unpublished) vacuoles as detected by light microscopy are distinct from the Golgi seen with the electron microscope. Chicken thrombocytes with both a Golgi and vacuoles, located at opposite poles, have been described using electron microscopic (EM) techniques (Daimon and Uchida, 1982). Presumably, vacuoles occur due to the accumulation of secretory products earlier assembled at the Golgi.

Reactive thrombocytes are often found in the same fields as CAB and free bacteria. Given the recognition of the phagocytic ability of thrombocytes and the importance of phagocytosis in bacterial defense, these observations are consistent with earlier *in vitro* observations (Carlson et al., 1968; Wigley et al., 1999). A monoclonal antibody (K1) described by Kaspers et al. (1993) reacted with the surface of both macrophages and thrombocytes. The K1-reactive surface receptor substance may function as an attachment site in the phagocytic response; a property shared by both cell types. The metastasis of mammalian tumors by extravasation is facilitated by platelets (Schlesinger, 2018). The elaboration of pseudopods (Figure 5A, B) is an early manifestation of the means where other (inflammatory) cells cloaked with thrombocytes begin the extravasation process.

Toroids as described here appear to challenge a suggestion that avian thrombocytes are less likely than mammalian platelets to form vaso occlusive emboli due to lower levels of $\alpha(_2b)\beta_3$ integrin (Schmaier et al., 2011). Toroids can become even larger than those in Figure 7A, B and therefore occlude limb vessels; predisposing to the development of lameness and other leg problems. Triggering receptors expressed on myeloid cells (TREM) family receptors are known to be expressed on the thrombocytes surface. These Ig-like molecules imbedded in the cm are thought to have soluble versions as well (Turowski et al., 2016). Toroids may result from binding of bacteria between the anchored Ig molecule with the soluble form adsorbed to the RBC surface (Figure 7, B).

Most reactive thrombocytes were accompanied by free or CAB. As duck embryos are about to begin the shell-pipping stage of incubation between Ed24 and Ed25, bacteria located on the shell could easily find an opportunity to gain entrance during this period. This would also account for bacteria and some of the reactive thrombocytes in hatchling samples. Moreover, there is an opportunity for bacteria to enter the egg shell and the egg proper through its pores at any time after the egg is laid. Moreso, if environmental conditions allow and good egg handling procedures are not in practice. Shell moisture can offer a vehicle for bacteria to move into pores.

Differentiation of a reactive thrombocyte from a normal physiologic variant may be problematic. As variation in thrombocyte morphology described here was invariably accompanied by reactive cells and atypia of another series (Cotter, 2021) the transition to a reactive state is likely a dynamic response only partially amenable to a microscopic study.

The occurrence of reactive thrombocytes in ducklings whose hemograms and H/L ratios are otherwise normal or unremarkable (Figure 8) draws additional attention to the weakness of relying strictly on a derivative statistic, an undefined H/L, to determine stress status (Lentfer et al., 2015).

CONCLUSION

The present observations suggest the analysis of the avian hemogram may benefit from including the condition of the thrombocyte as well as (atypical) cells of other series. This suggestion is justified because thrombocyte have roles in immunity beyond hemostasis. It is a cell that is often overlooked in determining stress levels or in evaluating the immune status of birds subjected to various experimental treatments. The accompaniment of activated/reactive thrombocyte by CAB and free bacteria should not be overlooked.

DECLARATION

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

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Competing interests: None.

Ethical considerations

Plagiarism, permission to publish, misconduct, data falsification and double publication or submission, and redundancy have been checked by the author.

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Effects of Date Seed Flour on Broiler Chickens' Growth Performance, Apparent Digestibility of Protein, and Apparent Metabolizable Energy

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ABSTRACT

The use of agricultural by-products as alternative feed ingredients is recommended to reduce production costs and maximize income. This study aimed to determine the effects of added date seed flour on broiler chickens' growth performance, protein digestibility, and metabolic energy. A total of 200 Lohmann MB-202 day-old chicks were randomly allocated to 5 treatments and 4 replication with 10 broiler chickens per cage. The treatments used in the current research included control without the addition of date seed flour (T0), basal feed + 2.5% date seed flour (T1), basal feed + 5.0% date seed flour (T2), basal feed + 7.5% date seed flour (T3), basal feed + 10% date seed flour (T4). The investigated parameters were growth performance, apparent digestibility of protein, and apparent metabolizable energy-nitrogen (AMEn). The result showed that adding date seed flour significantly affected final body weight, apparent digestibility of protein, and AMEn. In contrast, the date seed flour was no significant effect on the feed intake feed conversion ratio and income over feed cost. In conclusion, the addition of 10% date seed flour successfully increases final body weight, apparent digestibility of protein, and AMEn without any adverse effect on the broiler chickens.

Keywords: Broiler chicken, Date seed flour, Metabolizable energy, Performance, Protein digestibility

INTRODUCTION

Intensive poultry production systems are increasingly demanding a supply of protein. Generally, the success of poultry rearing depends on breed, feed, and management. Feed costs contributed to approximately 65-75% of poultry industry production (Sjofjan et al., 2021a). As farmers face the problem of the rising price of feed, it is crucial to use potentially available feed sources (Adli, 2021a). This case would be reducing the cost of poultry feeds as well as simultaneously contributing to reducing foot carbon print (Adli, 2021b). One potentially raw feed that can be used for broiler chickens is date palm (*Phoenix dactylifera* L.).

Dates are quite famous in Muslim countries, including Indonesia, as well as middle-east countries (Risa et al., 2018). In the Middle East, dates are the main staple food. In addition, the date palm is relatively adaptable to tropical areas, compared to subtropical areas. Thus, this condition would be advantageous for tropical countries like Indonesia. Dates consisted of minerals, such as iron, potassium, selenium, calcium, and vitamins (C, B_1, and B_2) (Primurdia and Kusnadi, 2014). The glucose content, crude protein, and crude fiber of date palms are around 50-57%, 1.8-2%, and 2-4%, respectively (Kresnadipayana and Lestari, 2017). Tareen et al. (2017) reported 7.5% fat and 5.8% protein content for date seeds. The amino acids consisted of 0.17% methionine, 0.31% lysine, and 0.36% threonine, which is higher than maize (Tareen et al., 2017).

The nutritional content of dates depends on their strain and level of maturity (Giovanny et al., 2019). Although dates are rich in nutritional content, the seeds should be processed to increase shelf-life, palatability, and digestibility. Accordingly, this study aimed to determine the effects of added date seed flour on growth performance, protein digestibility, and metabolic energy of broiler chickens

MATERIALS AND METHODS

Ethical approval

Ethical approval for the study was given by the Animal Care and Use Committee, University of Brawijaya, Indonesia No. 44-KEP-UB-2022.

Experimental design

A total of 200 MB-202 Lohmann chicks aged one day old (JAPFA Comfeed Ltd. Commercially) were randomly allocated to 5 treatments and 4 replicates of 10 broilers per cage. Each cage was adjusted with installed nipple drinks, free access water (*ad libitum*), rice hulls (130 × 100 cm, × 60 cm), and a controlled environment. The feed was given twice a day at the morning (07.00 a.m.) and afternoon (4 p.m.).

The treatments used in the research were the control without the addition of date seed flour (T0), basal feed + 2.5% date seed flour (T1), basal feed + 5.0% date seed flour (T2), basal feed + 7.5% date seed flour (T3), and basal feed + 10% date seed flour (T4). The investigated parameters included growth performance, apparent digestibility of protein, apparent metabolic energy (AME), and apparent metabolizable energy-nitrogen corrected (AMEn). The formulated feed (starter periods) consisted of maize, soybean meal, meat bone meal, corn gluten meal, crude palm oil, custom mineral mix, custom premix, and date seed (BSN, 2015a; Table 1). The finisher diet consisted of maize, soybean meal, meat bone meal, rice bran, crude palm oil, custom mineral mix, custom premix, and date seed (BSN, 2015b; Table 2).

Preparation of the date seed flour

The current study was conducted on dates (*Phoenix dactylifera* L.). The preparation step of making date seed flour was the following (Bouaziz et al., 2021). The mean weight of the dates was 25 g. First, the dates were subjected to reverse osmosis (RO) water. Afterward, dates were dried using the oven at 60°C and ground into a powder, followed by 3-day storage at room temperatures. Before beginning the trial, dates were taken for analyses of proximate (Sjofjan et al., 2021b).

Growth performance

The broiler chickens' weights were recorded at the beginning and the end of the weeks. In line, feed intake was measured as the differences between the amount of feed given and the remaining feed. Income over feed cost (IOFC) was calculated by considering final body weight multiples with broiler price at the site and differences between feed intake and feed price (Setyawan et al., 2019). In the end, the feed conversion ratio (FCR) was expressed by dividing the amount of feed given by the total weight gained (Adli, 2021b).

Apparent digestibility of protein and apparent metabolizable energy-nitrogen corrected

At the end of the experiments (35 days old), a total of 20 broiler chickens were removed into metabolic-artificial cages. The feed intake of the broiler chickens was recorded for apparent digestibility of protein on day 37 and AMEn on day 38.

Both AME and AMEn were measured according to the following formulae:

AME = AME-(ANR/FI)

AMEn = AME-(8.22×ANR/FI)

Where, ANR is apparent Nitrogen retention, FI denotes feed intake. The correction factor is 8.22 (Sjofjan et al., 2021a).

The apparent digestibility of protein is calculated using the following formulae.

Protein digestibility = (A X B) – (C X D)

(A X B)

Where, A is feed consumption, B signifies protein in feed, C accounts for the amount of excreta, and D denotes protein (%) in excreta (Regar and Kowel, (2021).

Statistical analysis

For the statistical analysis, analysis of variance (ANOVA) using a general linear model (GLM) was carried out using SAS OnDemand for Academics (ODA, Cary, NC, USA). The results were presented as standard error mean (SEM). Moreover, probability values were calculated using the least significant different testing. The following model was used:

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Yij signifies the parameters observed, μ is the overall mean, Ti denotes the effect level of date seed flour, and eij is the amount of error number. The treatments included control without the addition of date seed flour (T0), basal feed + 2.5% date seed flour (T1), basal feed + 5.0% date seed flour (T2), basal feed + 7.5% date seed flour (T3), basal feed + 10% date seed flour (T4). P value less than 0.05 was considered statistically significant. Moreover, probability values were calculated using the least significant different testing if there differ significantly ($p < 0.05$).

Table 1. Composition of broiler chicken diet at starter period

Ingredients (% as is basis)	T_0	T_1	T_2	T_3	T_4
Yellow maize	43.55	41.50	38.55	36.05	33.55
Maize gluten meal	22.45	22.45	22.45	22.45	22.45
Soya bean meal	25.00	25.00	25.00	25.00	25.00
Meat and bone meal 50	2.50	2.50	2.50	2.50	2.50
Poultry meat meal	2.50	2.50	2.50	2.50	2.50
Palm olein	1.00	1.00	1.00	1.00	1.00
Salt	0.15	0.15	0.15	0.15	0.15
Custom Mineral mix*	1.00	1.00	1.00	1.00	1.00
Custom Vitamin mix**	1.00	1.00	1.00	1.00	1.00
Date seed flour	-	2.50	5.00	7.50	10.00
Total	100	100	100	100	100
Calculated composition					
ME (Kcal/kg)	2982.00	2969.88	2957,77	2945,65	2933,54
Crude Protein (CP)	21.56	21.56	21.56	21.56	21.56
Crude fibre (CF)	3.60	3.78	3,97	4,15	4,34
Calcium (Ca)	3.0	3.0	3.05	3.11	3.05
Phosphorus (P)	1.5	1.5	1.5	1.5	1.5
Lysine	1.21	1.21	1.21	1.21	1.21
Methionine	0.70	0.70	0.70	0.70	0.70
Proximate composition					
(Wet Chemical analyses)					
ME (Kcal/kg)	2833	2873	2832	2811	2833
Crude Protein (CP)	21.01	21.00	21.00	21.00	21.00
Crude fibre (CF)	4.00	4.00	4.00	4.00	4.00
Calcium (Ca)	1.2	1.2	1.2	1.2	1.2
Phosphorus (P)	1.75	1.75	1.75	1.75	1.75

*: 12.5 mg Iron, 3 mg Copper, 37.5 mg Manganese, 31.32 mg Zinc, 5 mg Iodine, and 0.0625 mg Selenium; **: 6000 IU Vitamin A, 1000 IU Vitamin D3, 10 mg Vitamin E, 1.5 mg Vitamin K3, 5 mg Vitamin B1, 2.5 mg Vitamin B2, 0.5 mg Vitamin B6, 2 mg Vitamin B12, 5.5 mg Niacin, 0.2 mg Pantothenic acid, 30 mg Betaine; * T0: Control without the addition of date seed flour, T1: Basal feed + 2.5% date seed flour, T2: Basal feed + 5.0% date seed flour, T3: Basal feed + 7.5% date seed flour, T4: Basal feed + 10% date seed flour

Table 2. Composition of broiler chicken diet at finisher period

Ingredients (% as is basis)	T_0	T_1	T_2	T_3	T_4
Yellow maize	43.40	41.50	38.55	36.05	33.55
Maize gluten meal	19.45	19.45	19.45	19.45	19.45
Soya bean meal	25.00	25.00	25.00	25.00	25.00
Rice bran	9.00	9.09	9.09	9.09	9.09
Palm olein	1.00	1.00	1.00	1.00	1.00
Salt	0.15	0.15	0.15	0.15	0.15
Custom Mineral mix*	1.00	1.00	1.00	1.00	1.00
Custom Vitamin mix**	1.00	1.00	1.00	1.00	1.00
Date seed flour	-	2.50	5.00	7.50	10.00
Total	100	100	100	100	100
Calculated composition					
ME (Kcal/kg)	2983,70	2971,10	2971,10	2971,10	2971,10
Crude Protein (CP)	19.89	19.89	19,89	19,89	19,89
Crude fibre (CF)	4.30	4.49	4,49	4,49	4,49
Calcium (Ca)	2.0	2.0	2.0	2.0	2.0
Phosphorus (P)	0.5	0.5	0.5	0.5	0.5
Lysine	0.8	0.8	0.8	0.8	0.8
Methionine	0.3	0.3	0.3	0.3	0.3
Proximate composition					
(Wet Chemical analyses)					
ME (Kcal/kg)	2900	2900	2900	2900	2900
Crude Protein (CP)	19.00	19.00	19.00	19.00	19.00
Crude fibre (CF)	4.00	4.00	4.00	4.00	4.00
Calcium (Ca)	1.5	1.5	1.5	1.5	1.5
Phosphorus (P)	0.5	0.5	0.5	0.5	0.5

*: 12.5 mg Iron, 3 mg Copper, 37.5 mg Manganese, 31.32 mg Zinc, 5 mg Iodine, and 0.0625 mg Selenium; **: 6000 IU Vitamin A, 1000 IU Vitamin D3, 10 mg Vitamin E, 1.5 mg Vitamin K3, 5 mg Vitamin B1, 2.5 mg Vitamin B2, 0.5 mg Vitamin B6, 2 mg Vitamin B12, 5.5 mg Niacin, 0.2 mg Pantothenic acid, 30 mg Betaine; * T0: Control without the addition of date seed flour, T1: Basal feed + 2.5% date seed flour, T2: Basal feed + 5.0% date seed flour, T3: Basal feed + 7.5% date seed flour, T4: Basal feed + 10% date seed flour

RESULTS AND DISCUSSION

The results of adding date palm flour on growth performance can be seen in Table 3. The result showed that adding date seed flour had significant effects ($p < 0.05$) on final body weight, apparent digestibility of protein, and AMEn (Tables 3 and 4). In contrast, the date seed flour had no significant effect ($p > 0.05$) on the FCR and IOFC.

The feed intake results were lowest in T4, including 10% date seed flour. This result may be due to higher fiber in the date seed causing this condition. There is a difference between T_0 and T_4, the energy and protein, where one factor caused the result of this condition.

Table 3. The addition of date seed flour on the growth performance of broiler chickens

Parameters	T_0	T_1	T_2	T_3	T_4	SEM
FI (g)	2701.88	2675.81	2719.30	2705.47	2615.67	110.06
FBW (g)	1515.96^{b}	1500.00 ^{b}	1530.95^{b}	1531.72^{b}	1383.29^{a}	28.00
FCR	1.79	1.78	1.78	1.77	1.89	0.05
IOFC (IDR/head/day)	13127.22	13037.38	13445.98	13613.48	11271.36	1073.45

FI: Feed intake, FCR: Feed conversion ratio, FBW: Final body weight, IOFC: Income over feed cost. ^{a,b,c,d} Means with different superscripts in the row differ significant ($p < 0.05$). T_0: Basal diet, T_1: Basal diet + 2.5% date palm flour, T_2: Basal diet + 5% date palm flour, T_3: Basal diet + 7.5% date palm flour, T_4: Basal feed + 10% date palm flour

Table 4. The effects of date seed flour on protein digestibility and metabolic energy of broiler chickens

Parameters	T_0	T_1	T_2	T_3	T_4	SEM
Apparent digestibility of protein (%)	70.20	75.06	74.48	78.29	73.31	1.82
AME (Kcal/kg)	3659.32^{d}	3546.80^{c}	3294.25^{b}	3490.43^{c}	3161.67^{a}	61.25
AMEn (Kcal/kg)	3046.50^{d}	2891.52^{c}	2644.06^{b}	2806.96^{c}	2521.67^{a}	0.02

AMEn: Apparent metabolizable energy Nitrogen corrected, AME: Apparent metabolizable energy. ^{a,b,c,d} Means with different superscripts in the row different significant ($p < 0.05$). T_0: Basal diet, T_1: Basal diet + 2.5% date palm flour, T_2: Basal diet + 5% date palm flour, T_3: Basal diet + 7.5% date palm flour, T_4: Basal feed + 10% date palm flour

According to Allama et al. (2012), if the energy and protein do not meet standard criteria, the cell tissue would convert protein into energy. Moreover, the final body weight was optimum when date seed flour was given at 7.5%, while the body weight was at its lowest in T4 (10% date seed flour). Corollary, Masoudi et al. (2011) reported that date flour at a 10-30% level caused a reduction in final body weight. In addition, El-Faham et al. (2017) found that giving 10% date palm waste in feed reduced the final body weight. Accordingly, the use of date flour for more than 10% could have a significant effect on body weight. The reason can be the high crude fiber content of date seed flour. The capability of absorption is one factor on the growth performance instead feed intake and body weight (Astuti et al., 2015). Furthermore, FCR is the ratio of given feed weight over animal weight gain. An increase in FCR was indicative of inefficient feed used during the experiment. In addition, the replacement of corn with 15% date pits resulted in the FCR range of 1.72-1.84 (Hammod et al., 2018).

Income over feed cost can be reflected in the successful rearing management in the poultry industry. The IOFC in T3 was higher than in control and other treatments. According to Muchlis et al. (2021), IOFC is influenced by several factors, including feed intake, final body weight, and the selling price of broiler chickens. The FCR is key to the IOFC of broiler chickens (Prayitno et al., 2019).

The apparent digestibility of protein is one of the essential indicators related to the growth performance of broiler chickens (Azizah et al., 2020). The T_3 presented the best value for the apparent digestibility of protein. The appropriate value of the apparent digestibility indicated the high protein utilization in the broiler chickens. Furthermore, the higher result of apparent digestibility of protein can be reflected in the content of micro minerals. As El-Faki (2002) mentioned, date seeds consist of micro minerals, including iron (Fe) 7.4 mg/100 g, manganese (Mn) 2.8 mg/100 g, zinc (Zn) 1.9 mg/100 g, and copper (Cu) 1.2 mg/100 g. The Cu minerals had a main role in inhibiting microorganisms' activity in the digestive tract. Meanwhile, Zn minerals act as cofactors for protease enzymes that break down protein molecules into amino acids (Azizah et al., 2020). Natsir et al. (2018) reported that a reduction in protein digestibility causes low body weight in broiler chickens.

Metabolic energy measured in this study is AME and AMEn. The results indicated that the higher the use of date

seed flour in the feed, the lower the AME value (Table 4). This result may be due to the high crude fiber content in date seed flour which causes broiler chickens to require more energy to digest crude fiber. Feed ingredients with high crude fiber content can reduce the digestibility value of other feed ingredients because digesting the crude fiber content requires more energy (Noviadi et al., 2012). Therefore, the nutrient contents in date seed flour affected the nutrient content in experiments.

The AMEn result (T_4: Basal feed + 10% date palm flour) was lower than that of the AME (T_0: Basal diet + 0% control) caused by nitrogen retention in AMEn. Biologically, metabolic energy is determined by nitrogen retention (Sukaryana, 2010; Table 4). In addition, Dady et al. (2015) stated that high crude fiber will cause the availability of energy in the feed to decrease. The undigested crude fiber as well as other undigested nutrients, will excrete (Siabandi et al., 2018).

CONCLUSION

In conclusion, adding 10% date seed flour helps increase final body weight, apparent protein digestibility, and apparent metabolizable energy, without any adverse effect on the broiler chickens.

DECLARATION

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Author's contribution

Ilmiatus Sholichatunnisa contributed to collecting data, analysis of proximate, data analysis, and preparing the manuscript. Osfar Sjofjan contributed to the research design and supervision; Tri Eko Susilorini provided a sample and site for *in vivo* and supervision. Muhammad Halim Natsir was the supervisor. Danung Nur Adli supervised and revised the manuscript grammatically. All authors read and approved the final version of the manuscript in the present journal.

Competing interest

No potential conflict of interest relevant to this article was reported.

Ethical consideration

All authors have been checked the ethical issues, plagiarism, fabrication and/or falsification, double publication, and redundancy.

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Effect of Hybrid Nanomaterial of Copper-Chitosan against Aflatoxigenic Fungi in Poultry Feed

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ABSTRACT

In the past decades, the application of nanotechnology indicated significant improvements in animal health. In the present work, 60 samples of poultry feeds were examined, including 20 samples for each yellow corn, soya bean, and processed feed. The prevalence of total fungi was reported as 100%, 95%, and 100% in yellow corn, soya bean, and processed feed, respectively. Toxin-producing *Aspergillus flavus* represented 75% of isolates from yellow corn, 88% from soya bean meal, and 50% from processed feed. Aflatoxins were found in 88%, 60%, and 80% of yellow corn, soya bean, and processed feed with mean levels of 18.5 ± 3.216.0 ± 4.08.3 ± 1.7 ppm, respectively. The copper nanoparticles embedded with chitosan were green synthesized using an eco-friendly method, and their antifungal activity was evaluated against aflatoxigenic mold that recovered from poultry feeds. However, the molecular detection of virulent genes of *Aspergillus flavus* (*aflR* gene*)* after their exposure to high doses of copper-chitosan nanoparticles (CuCh-NPs) 150 µg/ml prevents *aflR* gene expression. The embedded chitosan with copper nanomaterial helps decrease their suspected toxicity to animals by reducing the used doses. Hence, the use of nanocomposites of nanomaterials with green benefits substances, such as chitosan, was the essential strategy of field application in veterinary.

Keywords: *Aspergillus flavus*, Chitosan, Copper nanoparticles, Nanotechnology, Poultry feed

INTRODUCTION

The use of nanotechnology is gaining more popularity in improving livestock health and productivity, especially in developing countries (Hassanen et al., 2019; Khalaf et al., 2019; Hassanen et al., 2020). Fungal infections caused by mycotoxigenic molds in food can cause significant carcinogenic effects on humans and animals (Hassan et al., 2022). Under adverse environmental conditions, aflatoxigenic molds produce aflatoxins (AFs) in food, and their consumption leads to several health problems (Adam et al., 2017; Çelik, 2020; Hassan et al., 2021). Given that the conventional methods of elimination, such as chemical antifungal, azoles, and antimycotoxins were proved inefficient, the elimination of AFs can be difficult and costly (Brunet et al., 2018; Di Mambro et al., 2019; Gintjee et al., 2020; Tiew et al., 2021). Consequently, recent studies have introduced new agents to eradicate toxin-producing pathogens (Singh et al., 2018). Earlier studies also assessed the advantages of using metal nanomaterials over chemical agents in controlling the growth and viability of pathogens (Tran and Webster, 2011; Mohd Yusof et al., 2021). In the same vein, others used copper and zinc nanoparticles (Cu-NPs and Zn-NPs, respectively) for the degradation of fungi and mycotoxins to improve the safety of food production (Castro-Mayorga et al., 2020; Agrimonti et al., 2021; Konappa et al., 2021; Hassan et al., 2022). Therefore, the present study aimed to evaluate the prevalence of AFs in feed and the effect of copper-chitosan nanoparticles (CuCh-NPs) on inhibiting the fungi and aflatoxin-regulating genes using molecular detection.

MATERIALS AND METHODS

Samples

A total of 60 samples of poultry feeds were examined, including 20 samples from yellow corn, soya bean, and processed feed. Approximately 100 g from each sample was aseptically collected from poultry farms from

June to August 2021 in sterile polyethylene stretch film followed by an ensiling process, and it was stored in a dry aerobic place.

Isolation and identification of fungi species in samples

To begin, 5 g of each feed sample was separately transferred aseptically into sterile tubes, to which 45 ml of sterile distilled water was added, and 10-fold serial dilutions were prepared. Afterward, 1 ml of the previously prepared serial dilutions was inoculated separately into sterile Petri dishes plates and mixed with Dichloran-rose Bengal chloramphenicol agar or Sabouraud's dextrose agar (SDA) medium containing 0.05 mg of chloramphenicol/ml. The plates were left to solidify and dry. They were then incubated aerobically in the incubator at 25°C ± 1°C for 5 days. The plates were read during 2-5 days of incubation. The fungal cultures were separated based on morphological characteristics, including colony size (diameter, millimeter), texture, and surface. The fungal cultures were examined periodically during the incubation period. The culture characteristics and sporulation on different culture media were recorded after 7 days of incubation at 28°C. The morphological characteristics of each fungal isolate were determined using the light microscope (OPTO-EDU, China). The microscopic examination of fungal isolates was described after the fungal colonies were sporulated on the different culture media. For this purpose, small mycelia part from the center and edge of the growing colony was mounted onto a microscope slide using distilled water and covered by a cover slip. The characteristics of vegetative and reproductive structures, such as hyphal color and structures, spore shape, as well as spore size, were determined (ISO 21527/1, 2008; Pitt and Hocking, 2009).

Copper nanoparticles

Copper nanoparticles with the size of 50 nm were prepared at Biochemistry, Toxicology, and Feed Deficiency Department, Animal Health Research Institute, Egypt, and identified at the Central Laboratory of Elemental and Isotopic Analysis, Nuclear Research Centre, Egypt.

Synthesis and characterization of chitosan-copper nanoparticles

The method was based on a study by Du et al. (2009), indicating some changes as copper ions were converted into nanosized material by mixing with a solution of acetic acid and chitosan, in which chitosan was dissolved in 1% (v/v) acetic acid to obtain a 0.3% (w/v) chitosan solution followed by refrigeration for 12 hours. The mixture was then centrifuged at 12000 rpm for 20 minutes at 4°C (Sigma Laborzentrifugen, Germany). The sediment was washed with distilled water, centrifuged again, and frozen until use. The freeze-dried CuCh-NPs were identified according to the method of Kaur et al. (2015). Their structures were detected by transmission electron microscopy (JEOL 2100F TEM instrument), and their infrared spectra were obtained using FTIR (Fourier transform infrared spectroscopy, Spectrum BX11, USA).

Preparation of tested isolates

The tested *Aspergillus flavus* (*A. flavus*) that were isolated from the present samples were subjected to Polymerase chain reaction (PCR) to identify their virulent genes. They were cultured on Sabouraud's dextrose broth medium and incubated at 25°C for 1-3 days for proper growth. The negative control was *Fusarium* species, while the positive control was standard isolates of *A. flavus*. On the other hand, the isolates of *A. flavus* were treated with CuCh-NPs under a septic condition in 100 ml flasks, then 20 ml of SD broth was added, and 0.2 ml of 10^4 spores was inoculated into the flask. The doses of treatments were leveled as low as 50 µg/ml and as high as 150 µg/ml for Cu-NPs. Then, the treated isolates were incubated at 25°C for 3 days and kept at 5-8°C until DNA extraction.

Genotypic evaluation of aflatoxigenic genes of *Aspergillus flavus*

DNA extraction and PCR amplification were performed according to Somashekar et al. (2004) and Fittipaldi et al. (2012). Genomic DNA of the strains was obtained using the genomic DNA Extraction Kit (Quick-DNA Miniprep DNA purification) following the manufacturer's instructions. DNA concentration was determined spectrophotometrically at 260/230 nm using SPECTRO star Nano BMG LABTECH. DNA was stored at -20°C until PCR amplification for the target fragments of aflatoxin-producing and control fungal genes. Invitrogen Company prepared the PCR primer used in the current study (Table 1). The PCR reaction was performed in a Gradient Thermal cycler (1000 S Thermal cycler Bio-Rad USA). The reaction mixture (total volume of 50 µl) was 25 µl Dream green PCR Mix (DreamTaq Green PCR Master Mix (2X) Thremoscientific Company, cat., No. K1081, USA), 5 µl target DNA, 2 µl of the primer

(containing 10 p mole/ µl), and the mixture was prepared by sterile Nuclease-free water to 50 µl. The PCR amplification conditions for the aflatoxin regulatory gene were 5 minutes for the initial step at 95°C, followed by 35 cycles at 95°C for 30 seconds, 56°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. Amplification products were electrophoresed in agarose gels in Tris-borate-EDTA (TBE) buffer with 1 µl of ethidium bromide/gel added for visualization under UV light (1.5% w/v, Agarose, Sigma, USA), using 100 bp DNA Ladder H3 RTU (Ready-to-Use) Cat. No. DM003-R500 from Gene Direx, Inc. Company, Litwania (Isalar et al. 2021).

Measurement of the minimum inhibitory concentration of CuCh-NPs against isolated *Aspergillus flavus*

The minimum inhibitory concentration (MIC) of CuCh-NPs for aflatoxigenic *A. flavus* was detected by a broth micro-dilution method (CLSI, 2008) which starts by adding 900 µl of Sabouraud's dextrose (SD) broth in plastic test tubes, then inoculating with100 µl of *A. flavus* (10^4 spores/ml), then adding 100 µl of CuCh-NPs in 0, 50, 100, 150 µg/ml concentrations, then incubated for 2-5 days at 25-28°C. The MIC that suppressed the growth of pathogen cultures and the turbidity was checked every 24 hours. A UV-vis spectrophotometer detected the optical density of each tube content (SP-LUV759, China) set at 405 nm.

Statistical analysis

Results were expressed as mean ± SE. The statistical analysis was conducted using Statistical Package for Social Sciences Version 14, released in SPSS (2006).

Table 1. Primers for molecular identification of *Aspergillus flavus*

Virulence factor	Target virulence gene	Primers	Amplicon size (Base pair)	Annealing temperature (°C)	Reference
Aflatoxin regulatory gene (*aflR)* of *A. flavus*	*aflR*	*afl*R-F AACCGCATCCACAATCTCAT *afl*R-RAGTGCAGTTCGCTCAGAACA	800	60	Somashekar et al. (2004)

RESULTS AND DISCUSSION

The results in Table 2 revealed that the prevalence of total fungi was 100% in yellow corn, 95% in soybean, and 100% in processed feed. *Aspergillus flavus* was the most prevalent mold of *Aspergillus* spp. with a total incidence of 45%. *Aspergillus* species were *Aspergillus ochraceus, A. niger, A. candidus, A. fumigatus, and A. glaucus*. The detected molds were *Penicillium* spp., *Mucor* spp., and *Rhizopus* spp., with a total incidence of 1.6%, 35%, and 33.3%, respectively. *Candida albicans* were found in 25% of samples. Similar findings were reported by FDA (2000), Hassan et al. (2020) who detected that *A. flavus* was the predominant species isolated from the feed. This proves that it requires several methods, such as using nanomaterials to inhibit fungal growth and activity from preventing human and animal diseases.

According to Table 3, the recovered *A. flavus* was used for the production of AFs, most of which were on yellow corn with the mean levels (750 ± 5.3 ppb) followed by processed feed (600 ± 6.1 ppb) and soya bean meal (170 ± 3.5 ppb). Toxin-producing *A. flavus* represented 75% of isolates from yellow corn, 88% from soya bean meal, and 50% from processed feed.

Generally, mycotoxins cause serious health hazards, especially in humid regions in developing countries, such as Egypt, due to the presence of AFs in feed (Nayak and Sashidhar, 2010; El-Nahass et al., 2019; Monda et al., 2020). Aflatoxins were detected in feed and feedstuffs by El-Hamaky et al. (2016) at levels ranging from 170 to 750 ppb.

Table 4 shows that AFs were found in 88% of yellow corn with a mean level of 18.5 ± 3.2 ppm; 60% of soya beans with a mean level of 16.0 ± 4.0 ppm, and 80% of processed feed with a mean level of 8.3 ± 1.7 ppm. Herein, the obtained results of AFs were more than the safe permissible limits and can cause serious adverse effects on health by causing hepatic injury and cancers (FDA, 2000). The results agree with the previous findings of Frisvad et al. (2006), Nayak and Sashidhar (2010), and Hassan et al. (2020), who detected the dangerous hepatic-carcinogenic effects of AFs on rabbits and rats' livers.

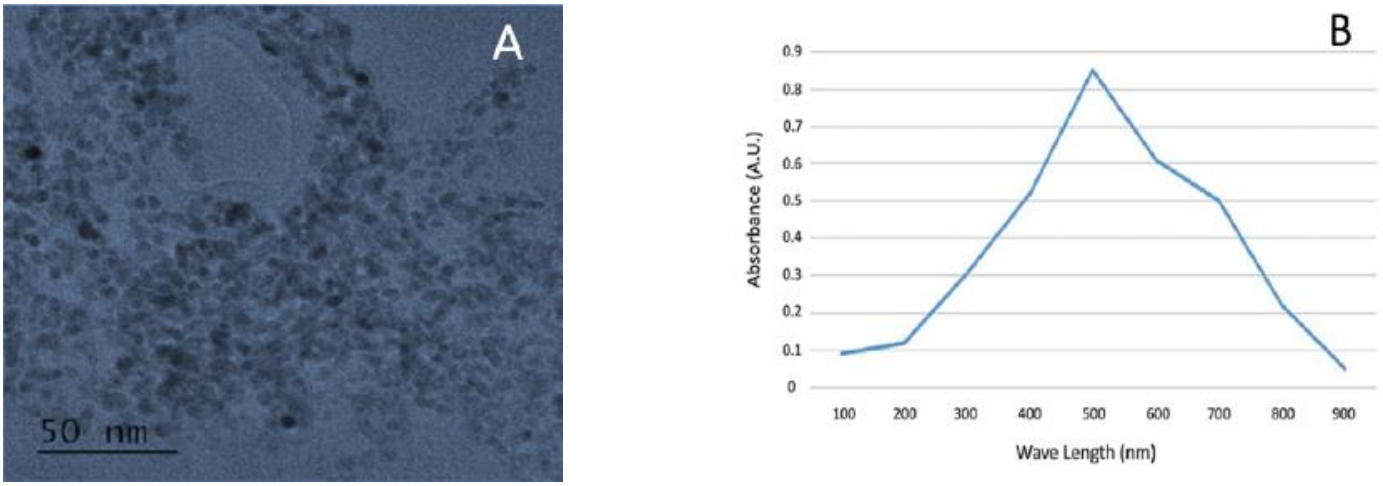

Figure 1. The morphological characters of copper-chitosan nanoparticles (CuCh-NPs) under transmission electron microscopy (50 nm in size, **A**). UV–visible spectrophotometry of CuCh-NPs (the peak was at a range of 500–600 nm, **B**).

These results in Figure 1 are similar to the finding of Vanti et al. (2020). Due to the emergence of multidrug-resistant bacteria, conventional antibiotics are rendered ineffective (Hassan et al., 2020a), which led to the use of nanosized materials as an alternative to the traditional antimicrobial drug (Abinaya et al., 2016; Munir et al., 2020). Furthermore, nanosized particles were more effective than crude metals (El-Sayed and Kamel, 2020).

Today, several microbial infections, such as fungal and bacterial infections, have a multidrug resistance to conventional antibiotics, increasing the severity of the conditions (Hassan et al., 2020a). Hence, the search for new effective antimicrobial agents is required, and the nanosized materials showed significant success in this purpose as Zn-NPs and Cu-NPs (Sharma et al., 2018; Zakaria et al., 2020). The nanomaterials are more effective than crude materials and can be used for antibiotic and disease diagnosis (El-Sayed and Kamel, 2020). They may be supplemented in drinking water and feeds of broiler chickens to improve their health and immune status (Hassanen et al., 2020; Hassan et al., 2022).

As can be seen in Table 5, the increase in the CuCh-NPs concentration led to a decrease in optical density (OD), degree of turbidity (DT), and growth after treatment (GT). Therefore, the minimum inhibitory concentration (MIC) of CuCh-NPs against *A. flavus* was determined to be 150 µg/ml. Nanomaterials can inhibit microbes by penetrating them and damaging their protein and DNA synthesis (Rudramurthy et al., 2016; Huang et al., 2020).

PCR detected the toxic gene (*aflR*) in *A. flavus* strains found in the samples. The efficacy of CuCh-NPs was evaluated the inhibiting that gene. Previous studies have also successfully detected *aflR* gene in *A. flavus* recovered from feed (Scherm et al., 2005; Cruz and Buttner, 2008; El-Hamaky et al., 2016). The exposure of *A. flavus* to high doses (150 µg/ml) of CuCh-NPs significantly decreased the *aflR* gene expression (the efficiency percentage, the molecular weight of DNA, and the cycle threshold of the gene declined). Currently, the *aflR* gene expression in the case of treatment with a low dose (50 ug/ml) also resulted in similar activity but lower than the exposure to high doses of CuCh-NPs (Table 6). Hence, the exposure of virulent genes of toxigenic fungus to high amounts of nanomaterials resulted in the complete removal of genes and prevented drug resistance. The present study indicated the high efficiency of CuCh-NPs nanocomposites in suppressing the viability and growth of aflatoxigenic *A. flavus*. Several studies reported that Cu-NPs have antimicrobial potential against isolated fungi from clinical cases of animal disease and feeds (Sharma et al., 2018; Zakaria et al., 2020; Hassan et al., 2022). Currently, the composites of metals nanomaterials with green benefits materials, such as the chitosan effect, decrease the used dose of nanomaterials and overcome its suspected ecotoxicity. Hence, nanotechnology has significant progressive advancements in biotechnology and biomedicine related to human and animal science as it increases the safety of their health and production (Contera et al., 2020).

Table 2. Incidence of fungi species in poultry feeds

Fungal species	Yellow corn (20)		Soya bean meal (20)		Processed feed (20)		Total (60)	
	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%
Total fungi	20	100	19	95	20	100	59	98.3
Aspergillus species	17	85	14	70	15	75	46	76.6
Aspergillus flavus	8	40	9	45	10	50	27	45
Aspergillus ochraceus	3	15	2	10	4	20	9	15
Aspergillus niger	6	30	5	25	7	35	18	30
Aspergillus candidus	4	20	2	10	2	10	8	13.3
Aspergillus fumigatus	3	15	1	5	1	5	5	8.3
Aspergillus glaucus	1	5	0	0	0	0	1	1.6
Penicillium species	0	0	0	0	1	5	1	1.6
Mucor species	7	35	5	25	9	45	21	35
Rhizopus species	5	25	8	40	6	30	20	33.3
Geotrichum species	1	5	1	5	0	0	2	3.2
Candida albicans	5	25	6	30	4	20	15	25

No +ve: Number of positive

Table 3. Production of aflatoxins B_1 by *Aspergillus flavus* isolated from poultry diets

Source of *Aspergillus flavus* isolates	Incidence of toxigenic *Aspergillus flavus* isolated from poultry feeds			Produced aflatoxins (ppb)	
	Total tested	No. +ve	Percentage	Mean levels	Types
Yellow corn	8	6	75	750 ± 5.3	B^1,B^2
Soya bean meal	9	5	88	170 ± 3.5	B^1, B^2, G^1 , G^2
Processed feed	10	5	50	600 ± 6.1	B^1
Total	27	16	59.2		

The permissible limits of AFB_1 were 15 ppb (WHO, 2002) and 20 ppb (FAO, 2004). No +ve: Number of positive.

Table 4. Levels of aflatoxins in poultry feeds

Feed types	Incidence of aflatoxins		Aflatoxins in samples (ppm)			Types of aflatoxins
	No. +ve	Percentage	Maximum	Minimum	Mean ± SE	
Yellow corn	22	88	30.0	9.5	18.5 ± 3.2	B_1, B_2, G_1,G_2
Soya bean meal	15	60	23.0	10.2	16.0 ± 4.0	B_1, B_2, G_1, G_2
Processed feed	20	80	3.2	1.6	8.3 ± 1.7	B_1, B_2, G_1, G_2

The permissible levels of aflatoxin, according to WHO (2002) 15 ppb and FAO (2004) 20 ppb. SE: Standard Error, No +ve: Number of positive

Table 5. Optical density and degree of turbidity of treated *Aspergillus flavus* at a gradual concentration of CuCh-NPs

CuCh-NPs concentrations (µg/ml)	*Aspergillus flavus*	
	OD (a.u)	DT and GT
0	2.27	4+
50	1.65	3+
75	1.38	2+
100	1.08	1+
125	1.00	1+
150	0.60	0

Control antifungal: Fluconazole 20 µg (Its OD: zero and turbidity: zero), OD: Optical density of treated spores at wavelength 405 nm, DT: Degree of turbidity of treated suspension, GT: Growth after Treatment. (a.u): absorbance unit

Table 6. Detection of *aflR* regulatory gene expression of *Aspergillus flavus* before and after treatment with CuCh-NPs

Aspergillus flavus	*aflR* gene expression at different doses of treatment					
	Eff. (%)		Mole		C.T	
	Low dose	High dose	Low dose	High dose	Low dose	High dose
Untreated controls	94.42		3.45		26.33	
Treated with CuCh-NPs	32.46	13.63	0.01983	0.0198	26.62	23.26

Eff: Efficacy of *aflR* gene expression, Mole: Molecular weight of DNA (µg/ml), CT: Cycle Threshold CuCh-NPs: 50 µg/ml (Low dose), 150 µg/ml (High dose)

CONCLUSION

The presence of aflatoxigenic molds in animal feeds can produce AFs. The essential preventive and therapeutic activities of Cu-NPs embedded with chitosan have been evaluated against the aflatoxigenic mold. Additionally, CuCh-NPs could remove *aflR* genes of *A. flavus* when a higher dose of 150 µg/ml was used. The conjugation of Cu-NPs with chitosan reduced the used dosages of metal nanoparticles and avoided the toxic hazard of copper nanoparticles. Therefore, more studies are needed to evaluate the effects of copper chitosan nanoparticles at different doses in poultry diets.

DECLARATIONS

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Authors' contributions

Noha Oraby, Rasha Sayed-ElAhl designed the research. All authors analyzed the data. Atef Hassan wrote the draft of the manuscript. Noha Oraby, Rasha Sayed-ElAhl and Manal El-mesalamy have revised the manuscript. All authors read and approved the last version of the manuscript for publishing in the present journal.

Competing interests

The authors declare that they have no competing interests.

Ethical consideration

The authors investigated ethical issues such as plagiarism, permission to publish, malfeasance, data falsification and/or fabrication, double publishing and/or submission, and redundancies.

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Proximate Chemical Analysis and Deterioration Criteria of Goose Giblets

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ABSTRACT

Goose meat is one of the most common types of meat consumed worldwide. Egyptian goose species, known as *Alopochen aegyptiacus* is one of the first reared poultry species. As meat consumption and the need for animal protein rise globally, edible giblets can serve as abundant protein and fat sources. Recently, edible giblets have become readily available, quick-to-prepare food on the market. This study aimed to reveal the proximate chemical composition (protein, fat, moisture, and ash) as well as the deterioration criteria (pH, Total volatile basic nitrogen [TVBN] value, and thiobarbituric acid reactive substance [TBA] value) of Egyptian goose giblets, including liver, gizzard, and heart. A total of 60 samples of goose giblets, including liver, gizzard, and heart (n = 20 each), were collected from Giza and Cairo cities, Egypt. The results showed a marked variation among each giblet type. The goose's highest protein content (24.48%), moisture content (72.42%), and fat content (12.18%) were recorded for liver, gizzard, and heart, respectively. Moreover, the highest pH (6.72) and TVBN mean value (5.61 mg/100 gm) were indicated in goose's livers, while the highest TBA mean (0.67 mg malonaldehyde/kg) was obtained from goose' hearts. These findings may provide a clear understanding for both consumers and possessors about the nutritional value of goose giblets which could be used as an alternative protein source. Moreover, the obtained data in the current study could help meat technology processors to add nutritional value to goose products using goose giblets.

Keywords: Chemical analysis, Deterioration criteria, Fat, Giblets, Goose, Protein, pH

INTRODUCTION

Meat consumption is rising globally due to population growth, so the way the earth will supply the world's population with the predicted amount of protein it will require in 2050 is a matter of concern (Nijdam et al., 2012; Aiking and de Boer, 2020; Limeneh et al., 2022). Protein originated from the Greek word "proteios", meaning principal or primary, so it fits nutrition as protein is a vital building block for human tissues (Wu, 2021). To sustain development and good health, humans require an adequate amount of high-quality nutritious protein (WHO, 2007). Daily protein intake for adults is about 1% of their body weight, half of which is recommended to be supplied from animal protein sources as it has more beneficial nutritional value than plant ones (Can and Can, 2022).

The Egyptian goose species (*Alopochen aegyptiacus*) is one of the first avian species that had been domesticated for more than 4000 years (Alagawany et al., 2020). It has the fastest growth rate among birds, reaching about 75% of adult weight within 9 weeks of rearing (Tilki et al., 2005; Ashour et al., 2020). Eviscerated goose carcass yields 75.5% and 65.9% of live bird weight with and without giblets, respectively. Therefore, about 10-15% of a bird's live weight is made up of giblets. (Sierra et al., 2022). Generally, edible poultry giblets consist of the liver, gizzard, and heart. However, the neck is not considered a part of the giblets if it is still connected to the carcass, according to Commission Regulation No. 543/2008. Nowadays, edible poultry giblets are offered in markets as a food product for consumption (Barker et al., 2004). Furthermore, the demand for edible poultry giblets

is increasing daily, especially due to their rapid preparation method and high nutritional value (Álvarez-Astorga et al., 2002; Nollet and Toldra, 2011).

Goose liver contains a high ratio of proteins that contain balanced amino acids; moreover, its extracted fat is healthy as it is rich in linoleic polyunsaturated fatty acid (Mitchell and Block, 1946; Li, 2018). Furthermore, edible goose liver, named "foie gras" is a popular French food ingredient made with goose liver fattened by force-feeding (Arroyo et al., 2017). Gizzard is where digestion takes place in poultry and is an edible poultry by-product rich in protein, iron, and zinc (Batah et al., 2012). Gizzard is a popular traditional snack food in Asia (Chang et al., 2013). In addition, the heart is also an edible poultry giblet that is rich in fat (Ho et al., 2008). Adding value to those edible internal organs (giblets) is a promising food technology trend to use these giblets in manufactured meat products, such as liver patties and pickled gizzard (Anandh et al., 2019).

Therefore, the current study focused on the chemical compositional analysis and the deterioration criteria (shelf life) of the edible Egyptian goose (*Alopochen aegyptiacus*) giblets regarding the previously published limited data on this subject.

MATERIALS AND METHODS

Ethical approval

The Faculty of Veterinary Medicine at Cairo University, Giza, Egypt, accepted the study design following the regulations and recommendations of the committee on animal welfare and ethics. There were no live animals utilized in the current investigation. All goose-edible giblet samples used in this study were collected from local markets as chilled products.

Sampling collection

A total of 60 chilled edible Egyptian goose giblets (*Alopochen aegyptiacus*), including livers, gizzards, and hearts (n = 20 each), were collected randomly from different markets all over Giza and Cairo cities (30 samples from each city), Egypt, from January to March 2022. Previously, giblet samples were freshly collected after the halal slaughter of goose, and evisceration occurred within 30 minutes in the commercial slaughterhouse. After Salvaging giblets, they were put in plastic bags. Samples were transferred immediately via cooled iceboxes within 1 hour to the Food Hygiene and Control Department Laboratory in the Veterinary Medicine Faculty, Cairo University, Egypt, to perform further analysis.

Sample preparation

Goose liver was trimmed of all excessive fat. Meanwhile, the gizzard was cut into halves with the removal of its content and inner cuticle layers. The heart was also opened, and the blood was washed out. After that, each giblet (liver, gizzard, heart) has been minced and mixed separately to obtain a homogenous representative sample for analysis. The time of sample preparation did not exceed one hour.

Physio-chemical analysis

Proximate compositional chemical analysis

Moisture, protein, extracted fat, and remaining ash content was evaluated by AOAC (2005). Moisture content was evaluated using 10 g of the prepared sample, which was taken in aluminum moisture cans -2-1/2 diameter and then put in a hot air oven (Heraeus UT6 Oven, Germany) at 103^{o}C for 16 hours until obtaining two successive weights. Protein content was evaluated by measuring the nitrogen content in the Kjeldahl digestion unit (VELP Scientifica F30110182 Model DK 6). Samples were digested using concentrated sulfuric acid at 420°C for 45 minutes. After that, distillation was performed on a steam distillation Kjeldahl unit (VELP Scientifica UDK 126D), and titration was done using 0.02N Hydrochloric acid. Finally, the conversion protein factor (6.25) was calculated. Fat was extracted using petroleum ether 20-40^{o}C in the Soxhlet extraction apparatus for 6-8 hours. Ash content was analyzed in a crucible containing a 5 g sample and then put in Muffle Furnaces (Thermolyne ™ IFD1540M-33, United States) at 550°C for 3.5 hours.

Deterioration analysis

At this stage, 5 g of the prepared giblet sample was homogenized with 20 ml of distilled water in Stomacher (Lab-Blender 400, Tekmar Corporation, England). The calibration of the pH meter (Lovibond Type 330) was done using chem lab buffer solution at pH 4.00 and 7.00. Then, the pH in the homogenate was measured by taking three reading for each sample, as described by Zaki et al. (2021). Thiobarbituric acid value was examined as described by Ali et al. (2007) in screw-capped tubes by adding 1 ml of sample homogenate, 1 ml Thiobarbituric acid (TBA), 1 ml Trichloroacetic acid (TCA), and 50 µl Butylated hydroxytoluene (BHT). The tubes were put in a boiling water bath for 15 minutes. Afterwards, they were cooled and centrifuged (Jouan Indust 220, France), then the absorbance of the supernatant was read at 531 nm on a

spectrophotometer (UNICO, SKU S-1200E, USA). Total volatile basic nitrogen.

Total Volatile Basic Nitrogen (TVB-N) value was measured following the distillation method as described by Kearsley et al. (1983)**,** in which the steam distillation Kjeldahl unit (Velp Scientifica UDK 126D, Germany) and Velp tube were used. The Velp tube contained 10 g of prepared giblet sample with 2 g of MgO and 150 ml of distilled water, while the receiving flask contained 25 ml of boric acid. After that, titration was carried out using 0.1 N sulfuric acid until the change of methyl red indicator color from blue to faint pink was considered the endpoint.

Statistical analysis

Descriptive statistical analysis was applied to the data collected using SPSS version 19.0 software to show the mean and standard error of the finding results. One-way ANOVA was used to compare the means of edible goose giblets, and the significance threshold was established at $p < 0.05$ using the least significant difference test (LSD).

RESULTS AND DISCUSSION

The proximate chemical analysis of the Egyptian goose's internal edible organs (giblets) is shown in Table 1. Among the examined giblets, extensive variations in the moisture content were detected, where the highest significant value was in the gizzard (72.42%, $p < 0.05$). In contrast, the lowest value (66.47%) was obtained from the heart. As reported by Abdullah and Buchtová (2022), the moisture content is higher in chicken giblets with percentages of 76.68%, 79.94%, and 77.36% for liver gizzard, and heart, respectively.

Table 1. Proximate compositional chemical analysis of edible Egyptian goose giblets

Item (g/100 g)	Liver	Gizzard	Heart
Moisture	70.34 ± 0.01^{b}	72.42 ± 0.02^{a}	66.47 ± 0.02^{c}
Protein	24.48 ± 0.01^{a}	24.11 ± 0.02^{a}	20.46 ± 0.02^{b}
Fat	3.63 ± 0.02^{b}	2.20 ± 0.02^{c}	12.18 ± 0.02^{a}
Ash	1.49 ± 0.02^{a}	1.27 ± 0.01^{a}	0.84 ± 0.02^{b}

Data in the table includes Mean ± standard error. ^{a,b,c} Within the same row with different superscripts are significantly different ($p < 0.05$).

Furthermore, results of protein analysis showed that goose liver and gizzard had a significantly ($p < 0.05$) higher protein (24.24 and 24.11 g/100 g, respectively) than the heart (20.46 g/100 g, Table 1). Compared to the obtained results in the current study, the chicken giblets analyzed by Abdullah and Buchtová (2022) indicated lower protein content in the liver (17.70%), gizzard (17.26%), and heart (13.83%). Although both goose meat and the gizzard are classified as muscles, the difference is that the first is striated, and the last is smooth in muscle fiber (Tokunaga et al., 2022; Wu et al., 2022). The mean protein value of the gizzard (24.11 g/100g) in the current study was higher than those reported by Geldenhuys et al. (2013) in the breast (20.18%) and the thigh (19.44%). However, the protein value can reach 22.3% in goose meat (Ding et al., 2014). Zouari et al. (2011) revealed that the protein content in turkey liver was relatively close to that of present findings, as turkey liver showed a protein value of 21.90%. Moreover, the goose giblet's protein is significantly higher than those found in chicken or duck giblets (Seong et al., 2015; Abdullah and Buchtová, 2022). This makes goose giblets a promising alternative protein source.

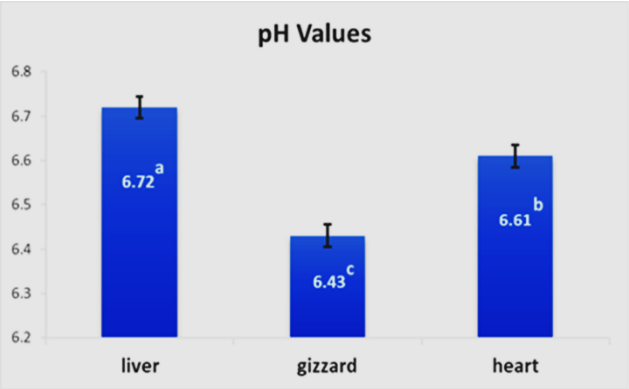
Regarding the fat content presented in Table 1, the fat content of the liver and gizzard had significantly low values of 3.63 g/100 g and 2.2 g/100 g, respectively ($p < 0.05$). Similarly, Zouari et al. (2011) reported a fat content of 2.9% in turkey liver. The highest significant fat content was obtained from the goose heart (12.18 g/100 g), which was probably due to the presence of coronary fat in the homogenized heart minced samples ($p < 0.05$). Moreover, the results of Ash analysis showed that goose liver and gizzard had a significantly ($p < 0.05$) higher ash content (1.49 and 1.27%, respectively) than the heart (0.84 g/100g, Table 1).

Poultry by-products are highly perishable; therefore, it is important to determine their freshness (Ozdemir and Yetilmezsoy, 2020). Freshness parameters, including TVBN and TBA values, are biomarkers for both protein and fat degradability (Mottram, 1998; Li et al., 2019). The pH value might serve as a guide for the early stages of decomposition (Yamanaka et al., 1987). Results shown in Graph 1 revealed that the pH mean value was significantly ($p < 0.05$) higher for the liver (6.72), compared to the heart (6.61) and gizzard (6.41), which could be regarded as the high glycogen content stored in hepatocytes (Baycumendur and Ergün, 2022).

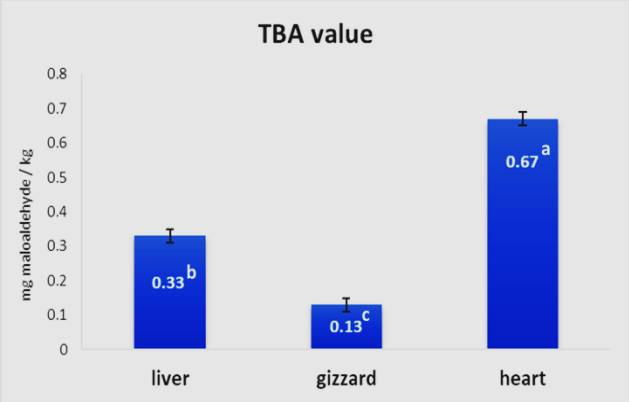
Freshness TBA value measures malonaldehyde, a secondary product of oxidative rancidity (Dahle et al., 1962; Pryor et al., 1976). Generally, a low TBA value gives a good integration of the shelf life of food items. As outlined by Egyptian standards, the TBA limit should not

exceed 0.9 mg malonaldehyde/kg of the sample (EOS 1090/2019). As can be seen in Graph 2, TBA value of edible internal goose giblets was significantly higher ($p < 0.05$) in the heart (0.67 mg mal/kg), compared to the liver (0.33 mg mal/kg), and gizzard (0.13 mg mal/kg). The freshness TBA parameter had the highest significant value in goose hearts ($p < 0.05$) as there was a strongly linked relationship between TBA value and fat content (Table 1). The unsaturated type of fat is more prone to oxidize and undergo rancidity (Ratrinia and Komala, 2022; Shehata et al., 2022). Mohamed et al. (2017) reported that chicken giblets' TBA is higher than TBA in goose giblets.

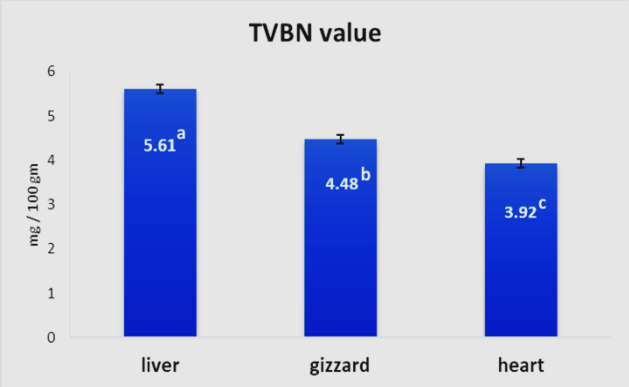
Freshness TVBN test measures the destruction of protein and releasing ammonia and its derivatives nitrogenous compounds, resulting from transamination or decarboxylation, which are returned to bacterial or related enzymes (Fan et al., 2009; Hopkins and Geesink, 2009; Khulal et al., 2016). The freshness TVBN limit should not exceed 20 mg/100 g of the sample as outlined by Egyptian standards (EOS 1090/2019). Graph 3 shows the TVBN of the Egyptian goose' edible giblets. The goose liver showed the highest value (5.61 mg/100 g), followed by the gizzard (4.48 mg/100 g), and the least value was in the heart (3.92 mg/100 g). The elevation in TVBN in the liver may be associated with endogenous enzymatic activity. The obtained TVBN values in this study were different from the results of chicken giblets explained by Mohamed et al. (2017) as they reported liver, gizzard, and heart TVBN values of 13.3 mg/100 g, 14.61 mg/100 g, and 14.87 mg/100 g, respectively. This difference could be attributed to the species variation.



Graph 1. Mean values of pH in edible Egyptian goose giblets ([a,b,c] Values within columns with different superscripts are significantly different ($p < 0.05$)).



Graph 2. Mean values of thiobarbituric acid in edible Egyptian goose giblets ([a,b,c] Values within columns with different superscripts are significantly different ($p < 0.05$).



Graph 3. Mean values of total volatile basic nitrogen in edible Egyptian goose giblets ([a,b,c] Values within columns with different superscripts are significantly different ($p < 0.05$).

CONCLUSION

Goose giblets can be considered a high protein source instead of other poultry species as well as it has low-fat content, making them an excellent nutritional source. The obtained data in the current study could be used as a reliable index for identifying the proximate chemical composition of edible giblets of the Egyptian goose (*Alopochen aegyptiacus*). Moreover, this study presents new insight into the Egyptian goose's shelf-life indicators for edible giblets since the previously published data on goose giblets is very scarce. Finally, it is recommended to maximize the benefits of goose giblets by considering them in processing goose food products.

DECLARATIONS

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Authors' contribution

Zeinab Mohamed Nagy performed the practical laboratory test. Mohamed Mohamed Talaat Emara supervised the work and designed the study. Nabil Abdelgaber Yassien supervised the work and revised the manuscript. Hamdy Mohamed Bakry Abdelhady Zaki: supervised the work, designed the experiment, and data analysis, revised the final manuscript version, and handled the correspondence for the publication process. All authors read and approved the final version of the manuscript for publishing in the present journal.

Competing interests

According to the researchers, the contents of this study are not influenced by or subject to bias due to any financial conflicts of interest or conflicts with other persons or organizations.

Ethical consideration

Ethical issues, all Authors have reviewed and approved the manuscript for ethical concerns, such as plagiarism, misconduct, data fabrication, and redundancy. Authors confirm that the data is original and have not been published elsewhere.

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The Impacts of Locally Cultivated Herbs on Physical Parameters and Meat Quality of Broiler Chickens

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ABSTRACT

Herbs greatly influence broiler chickens' performance and may alternate the use of antibiotics in the poultry sector. The study investigated the effects of local natural herbs on Ross 308 broiler chickens' physical characteristics and meat quality. A total of 702-day-old broiler chickens were divided into two trials consisting of 13 treatment groups fed 11 diets. Treatments included control groups (CGIa and CGIb); Basal diet (BD) free of antioxidants and antibiotics, and CGIIa, CGIIb; BD complemented with antioxidants and antibiotics, and experimental groups (EG); EGIII (1% peppermint + BD), EGIV (1% thyme + BD), EGV (0.5% peppermint + 0.5% thyme), EGVI (1% rosemary + BD), EGVII (1% chamomile flowers + BD), EGVIII (0.5% rosemary + 0.5% chamomile), EGIX (1% onion powder + BD), EGX (1% garlic powder + BD), and EGXI (0.5% onion powder + 0.5% garlic powder). Maximum and minimum feed intake averages were in EGV and EGIII (94.90 and 77.74 kg/group, respectively). Live body weight gains of both EGVI and CGIIa were significantly higher than EGIV, EGVIII, and CGIa. Chicks of EGVIII showed the lowest net weight percentage in relation to live body weight (carcass yield). Breast meat pH ranged between 5.26 and 6.14 (in EGVII and EGIV, respectively) 24 hours after cooling, and between 5.86 and 6.11 (in CGIa and EGVI, respectively) one month after freezing. Breast meat lightness was significantly higher in EGVI than EGVIII at 24 hours after cooling, and it was the highest in EGVI 1 month after freezing. Breast meat redness was the highest in CGIIa at 24 hours after cooling. EGIX showed a significantly higher redness value one month after freezing than EG X, CG Ib, and CG IIb. Yellowness ranged between 7.58 and 13.54 for EGX and CGIa, respectively, 24 hours after cooling, and between 8.29 and 13.95 a month after cooling for EGX and EGV, respectively. Tested herbs had comparable effects to antibiotics on chicken growth and meat quality. Rosemary (1%) had an ameliorative effect on chickens' body growth. Chamomile (1%) as well as thyme and peppermint mix (0.5% each), improved the palatability for feed.

Keywords: Broiler chicken, Chamomile, Garlic powder, Onion powder, Peppermint, Rosemary, Thyme

INTRODUCTION

The poultry industry's current goals are to improve the well-being of chickens and reduce the negative environmental effects. Regarding price and organoleptic qualities, broiler meat ought to be of adequate nutritional quality and palatable to the majority of consumers. Chick's growth and/or laying capacity augmented, the diseases were reduced and prevented, and the feed exploitation improved due to the use of feed additives. However, these additives were mainly antioxidants, antimicrobials, emulsifiers, enzymes, and pH control agents in poultry diets. Criticism is being directed at the use of antibiotics as growth enhancers, as reported by Iji et al. (2001) and Issa and Abo Omar (2012). Antibiotic resistance limits the use of antibiotics in microorganisms and drug residues in meat (CAFA, 1997). The elimination of antibiotics from chicken diets led to poor performance and increased susceptibility to diseases. Therefore, efforts were undertaken to discover other solutions to these problems. The European Union (EU) has also prohibited the use of antibiotics as growth promoters (Castanon, 2007), and research was done to find effective alternatives

for use in chicken. In case of poor management, herbs and herb extracts showed promise as natural feed additives and alternatives in flocks (Cross et al., 2003; Lewis et al., 2003; Hernandez et al., 2004) and have antioxidative and antibacterial effects on animals (Odoemelam et al., 2013). For instance, rosemary (*Rosemarinus officinalis*) has antimicrobial properties and stimulating effects on the digestive system (Al-kassie, 2008). The presence of thymol and carvacrol Thyme (*Thymus vulgaris*) has antibacterial and antioxidant properties (Wareth-Abdel et al., 2012). Active compounds of chamomile (*Matricaria chamomilla*) flowers increase resistance against microorganisms and improve final body weight gain (Mahmmod, 2013). Peppermint (*Mentha piperita*) added to basal diets enhanced feed conversion and broiler appetite (Ocak et al., 2008). Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) plant parts contain compounds that have antibacterial and antifungal properties (Goodarzi, 2013). Poultry production is one of the major components of the Lebanese agricultural sector (Darwish, 2003), and antibiotic use is needed to reduce production costs and produce healthier meat to meet European standards. Therefore, the current research aimed to investigate the effects of feeding broiler chickens with peppermint meals, rosemary, chamomile, thyme, garlic, and onion powders on performance, carcass traits, breast cuts, and physical quality during the growing period.

MATERIALS AND METHODS

Ethical approval

The research was approved by the Bioethics Committees of the Lebanese University, Faculty of Agriculture, Department of Animal Production, and the University of Forestry, Sofia, Bulgaria. and strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of November 24, 1986.

Population under test

A total of 702 one-day-old unsexed broiler chickens (Ross 308 breed) were used for 31 days in an opened poultry house in Lebanon. From day one to day 10 of age, chicks were placed under an artificial gas brooder located at a height not exceeding 1 m above floor level. A circular cardboard guard of 40 cm in height was used to keep the chicks inside. Chicks were receiving continuous light for 24 hours. The house was supplied with 75W Tungsten pulp lamps and adjusted to achieve light intensity between 5-10 lux at floor level.

The experiment consisted of two trials conducted at two different timings; the first trial on 432 chickens and the second trial on 270 chickens. In both trials, chicks were exposed to the same experimental conditions. The first trial investigated the effects of peppermint, thyme, chamomile, and rosemary, whereas the second one studied those of garlic powder and onion powder. In the first trial, chicks were randomly distributed among eight separate groups (treatments) of 54 chicks each and three replicates per group (18 chicks in each replicate). In the second trial, they were divided into five distinct groups (treatments) of 54 chicks each and three replicates per group (18 chicks in each replicate). Each group was provided approximately 5 m^2 of floor space.

All chicks were vaccinated against Gumboro disease (at days 10 and 20) via drinking water within two hours after the chickens were kept thirsty (Nobilis® Gumboro D78, MSD, Netherlands). Moreover, chickens were vaccinated against Infectious Bronchitis disease (Nobilis® IB Ma5, MSD, Netherlands) via drinking water (on day 15) and against Newcastle disease (Newcastle Disease Vaccine N-63, MSD, Netherlands) via drinking water (on day 20).

Feeding at starter period

During the starter period, all chicks received the same starter basal ration of commercial broiler starter diet (230 g CP/kg and 13.1 MJ ME/kg, Table 1) labeled and guaranteed by the manufacturer based on yellow corn-soybean meal mixture mash for 19 days (starting period) with neither addition of antibiotics and antioxidants nor herbs and spices.

Feeding at grower period

From day 20 to day 31, chicks were fed a commercial broiler grower diet (210 g CP/kg and 13.4 MJ ME/kg, Table 1) supplemented or not with the predefined herbs. Basal diets (BD) were formulated to meet minimum nutrient requirements established by the NRC (1994). Therefore, trial one consisted of eight treatment groups, including control groups (CGIa: BD free of antioxidants and antibiotics, and CGIIa: BD supplemented with antioxidants and antibiotics), and experimental groups (EGIII: 1% peppermint + BD, EGIV: 1% thyme + BD, EGV: 0.5% peppermint + 0.5% thyme, EGVI: 1% rosemary + BD, EGVII: 1% chamomile flowers + BD and EGVIII: 0.5% rosemary + 0.5% chamomile).

Trial II consisted of five groups, including control groups (CGIb: BD free of antioxidants and antibiotics, and CGIIb: BD supplemented with antioxidants and antibiotics) and experimental groups (EGIX: 1% onion powder + BD, EGX: 1% garlic powder + BD, and EGXI: 0.5% onion powder + 0.5% garlic powder).

Recorded indicators

Live body weight gain (LBWG) of chicks was recorded every time the feeding phase was changed using a digital balance (± 0.01g): at 19 days of age, marking the end of the growing period, and at 31 days, referring to the end of the finishing period on the total number of chicks of each group, then the average value of LBWG was calculated for each group. Feed intake (FI) was recorded to calculate feed conversion ratio (FCR) as follows: FCR=FI/LBW.

From each replicate of each group, three chicks aged 31 days of age were selected and left to fast for 24 hours and then weighed before and after slaughter, where they were left to completely bleed, after which the feather was manually plucked, and the troops eviscerated to obtain the net weight to live body percentage (Ragaa et al., 2016).

Post mortem breast muscle pH was measured at the two-time interval, 24 hours after cooling (4ºC) and a month after freezing at (-5ºC) using a portable pH meter (Adwa AD 131 Ph/mV Meter), equipped with a Piercing Tip Micro Probe. Each sample was measured in triplicate by direct insertion of the probe at 1-inch-deep in the muscle, and the average pH value was calculated for each treatment.

A whole piece of breast meat was evaluated for color in triplicates at 24 hours after cooling (4ºC) and 1 month (-5ºC) after freezing using the Hunter Lab colorimeter (ADCI-60-C instrument). The color of the breast meat was evaluated using the CIE color system, including L* (lightness), a* (greenness and redness), and b* (blueness and yellowness). All measurements were carried out on the surface of the scallop, in an area free of color defects (bruises, blood spots, and hemorrhages).

Left raw breast muscles were individually weighed at 24 hours post mortem, then they were reweighed after blotting dry by a paper towel. The samples were then placed in sealed polyethylene plastic bags, vacuum-packaged, and stored in a chiller at 4°C. The drip loss was calculated as a percentage relative to the initial muscle weight.

Drip loss (%) = ([Weight before treatment - weight after treatment] / weight before treatment) x 100.

Statistical analysis

One-way analysis of variance was conducted using "statistical 10 software" to evaluate significant differences between treatments and replicate means. The significance level was set at $p < 0.05$. The means (X) and standard deviation are used to display the results (SD).

Table 1. Recommended composition and nutrient content of the basic commercial diet

Nutrient	Starter diet (0-19 days of age)	Grower diet (20 to 31 days of age)
Energy (Kcal)	3100	3200
Protein (%)	21.5	19.5
Calcium (%)	0.87	0.79
Phosphorus (%)	0.435	0.395
Sodium (%)	0.16-0.23	0.16-0.20
Methionine (%)	0.51	0.47
Methionine+ cysteine (%)	0.99	0.91
Lysine (%)	1.29	1.16
Vitamin E (mg)	65	55
Vitamin B2 (mg)	6.5	5.4
Vitamin B12 (mg)	0.017	0.011

The diet is balanced based on NRC (1994), and recommendations of Ross 308 guideline (Aviagen, 2014)

RESULTS AND DISCUSSION

Daily observations revealed no health issues with the chicks. The chickens had strong legs, good size, and no signs of illness or inadequate nutrition. Among the 702 studied chickens, a total of 55 (7.83%) mortalities were recorded. However, a remarkable mortality rate was recorded in EGX (25.45%), where the diet fed to the chickens was supplemented with garlic. EGVII, EGVIII, CGIb, and CGIIb showed the lowest mortality rate (1.82%), followed by EGV (3.64%), EGIII, EGIV and CGIa (5.45%), CGIIa (7.57%), EGVI (10.91%), EGXI (12.73%), and EGIX (14.55%). Since all chickens were fed the same basic diet free of antibiotics and antioxidants, management and breeding practices can be blamed for the mortality rate. According to Yassin et al. (2009),

management parameters adopted at breeder farms, including the breeder age, strain, and feed company, and at hatcheries, such as the management of egg storage and hatching, are related to mortality at broiler farms. Besides, the quality of the day-old broiler directly impacts a chick's chance of survival. Different factors affect the quality of day-old chick, such as breeder age and genetic line, egg storage conditions and weight, and incubation conditions (Decuypere et al., 2001; Tona et al., 2004).

The collected data showed that combining peppermint with thyme (EGV) resulted in the highest FI (94.90 kg/group, Table 2). Feed intake was also high (92.29 kg/group) when the chickens were fed with chamomile (EG VII). Moreover, chickens of groups EGIII, EGIV, and EGX showed the lowest values of FI (77.74, 77.87, and 77.89 kg/group, respectively). Feed conversion was inferior in EGX (1.72) and EGXI (1.68), compared to the remaining groups. The most effective FCR values were in EGIV (1.29) and EGVI (1.3). At the end of the starter period (19 days old), the average feed intake (FI) of the chickens was 657.14 g/chicken. The chickens' appetite was consistent among all groups.

Table 2. Feed intake and feed conversion ratio at 1-31days of age in Ross 308 broiler chickens

Group	Feed intake (kg)	Feed conversion ratio
EGIII (1% peppermint + BD)	77.74	1.36
EGIV (1% thyme + BD)	77.87	1.29
EGV (0.5% peppermint + 0.5% thyme)	94.90	1.59
EGVI (1% rosemary + BD)	83.02	1.30
EGVII (1% chamomile flowers + BD)	92.29	1.63
EGVIII (0.5% rosemary + 0.5% chamomile)	88.21	1.53
EGIX (1% onion powder + BD)	80.92	1.50
EGX (1% garlic powder + BD)	77.89	1.72
EGXI (0.5% onion powder + 0.5% garlic powder)	86.92	1.68
CGIa (BD free of antioxidants and antibiotics)	88.49	1.54
CGIb (BD supplemented with antioxidants and antibiotics)	88.35	1.49
CGIIa (BD free of antioxidants and antibiotics)	82.89	1.51
CGIIb (BD supplemented with antioxidants and antibiotics)	79.69	1.41

BD: Basal diet, CGI: Control groups in trial I, CGII: Control groups in trial II, EG: Experimental group

These findings are at odds with those of Langhout (2000), Kamel (2001), Williams and Losa (2001), and Hernandez et al. (2004), who claimed that increased feed intake is attributed to the enticing effects of borneol, the active ingredient of rosemary. However, Panda (2005), Santurio el al. (2007), and Windisch et al. (2009) supported the findings of the current study by reporting that chamomile enhances FI because of its active components; coumarin glycosides, azulene, flavonoids, and fatty acids possess sedative, anti-inflammatory, antiseptic, and carminative activities. Improvement of LBWG and FCR for chickens fed diets containing one percent each of peppermint and rosemary was consistent with those reported by Al-Kassie (2008). It also supports the findings of Ertas et al. (2005), who found that adding 200 ppm of an essential oil mixture made of oregano, clove, and anise to broiler feed increased LBW and FCR in comparison to control groups. Rahimi et al. (2011) claim that thymol, carvacolo, borneol, and geraniol, the volatile components of thyme, increase enzymes and endogenous hormones' secretion, which in turn affect broiler performance when plant extracts are added (amylase and chymotrypsin). This will increase the intestine's absorption rate, which will benefit the FCR (Feizi et al., 2013). According to Amouzmehr et al. (2012), the broilers' performance in terms of feed intake, weight gain, and feed conversion ratio were unaffected by the addition of thyme. According to Goodarzi et al. (2013), diets supplemented with onions decreased feed conversion compared to those supplemented with antibiotics.

The highest numerical results concerning LBWG (Figure 1) were obtained in EGVI supplemented with rosemary and CG IIa, where antibiotics and antioxidants were added to the basal diet. LBWG results of both groups were significantly higher ($p < 0.05$) than EG IV (1061.41 ± 63.82 g), EG VIII (1048.3 ± 30.36 g), CG Ia (1073.1 ± 191.25 g). This may be because the active substances in rosemary green meal inhibit the overgrowth of harmful intestinal microorganisms and increase the activity of the thyroxin hormone, which speeds up the biochemical reactions and metabolites of nutrients (Mahmmod, 2013). These effects have a positive impact on the health and productivity of poultry. This confirms the findings of Kolacz et al. (1997), Al-Kassie (2008), Osman et al. (2010), and Sarker et al. (2010), which demonstrated an improvement in LBW as a result of the primary ingredients of herbs and essential oils, responsible of the majority of the antimicrobial activity (Abaza, 2003; Cross et al., 2007). Additionally, Spernakova et al. (2007) observed that the addition of rosemary powder at 500 mg/kg in the poultry diet resulted in a larger body weight gain.

Carcass yield in EGVIII was significantly lower ($p < 0.05$) than in all remaining groups (around 63.77%, Figure 2). For the edible organs and carcass yield, the result of this experiment matched those of Sarica et al. (2005), indicating that the effects of the supplementation of thyme powder did not significantly differ on the weights of internal organs, such as heart and liver.

Breast meat pH 24 hours after cooling and one month after freezing were assessed for all the studied groups, and the results are reported in figures 3 and 4, respectively. There was a remarkable significant difference ($p < 0.05$) in meat pH 24 hours after cooling at the level of EGVII whose chickens were fed a basal diet supplemented with chamomile, with pH (5.26 ± 0.16) being the lowest among all groups. It is well-known that chicken carcasses' pH changes during rigor mortis. Glycolysis, lactic acid production, and muscle oxygen liability reduction all contribute to the abrupt pH drop. This outcome is consistent with Schreurs (2000) findings. According to Olivo (1999), a low rate of pH fall throughout the slaughter process shows that the animals are not under stress, which is frequently correlated with increased meat softness (Ali et al., 1999). As shown in Figure 4, there were no significant differences in pH across the tested groups a month after freezing, which is consistent with the research conducted by Sang-Oh et al. (2013), who found that the pH of chicken flesh did not alter significantly among groups fed various herb extracts.

Lightness (L*), redness (a*), and yellowness (b*) were all assessed for the experimental groups 24 hours after cooling and a month after freezing.

Lightness measured 24 hours after cooling of EGVIII (51.13 ± 2.76) whose chickens were fed with a basal diet supplemented with a combination of rosemary and chamomile was significantly lower ($p < 0.05$) than the lightness of EGVI where rosemary was added only to the basal diet of the chickens (58.43 ± 1.9, Figure 5). After 1 month of freezing, EGVI showed a high L* value (53.43 ± 1.73), significantly differing from EGX (45.9 ± 0.43), EG XI (46.46 ± 0.58), CG Ib (46.24 ± 0.66), and CG IIb (46.32 ± 0.57, Figure 6). Moreover, EG X and EG XI showed significantly lower L* values (4.59 ± 0. 43; 46.46 ± 0.58), respectively, as compared to EGVI (53.43 ± 1.73), CGIa (52.33 ± 0.07), and CGIIa (51.62 ± 0.17). In addition, CGIa (52.33 ± 0.07) and CGIIa showed higher significantly different L* values than CGIb (46.24 ± 0.66) and CGIIb (46.32 ± 0.57, $p < 0.05$).

Obtained results of redness measured at 24 hours post mortem did not differ significantly among all the treatments ($p < 0.05$, Figure 7). However, the highest redness value was obtained in the positive control group CG IIa (8.33 ± 0.1), whereas the lowest values were in EGVII (6.39 ± 0.5) and EGVIII (6.39 ± 0.2). The chickens of EGIX showed a significantly higher ($p < 0.05$) a* value (9.91 ± 0.91) than EGX (6.57 ± 052), CGIb (7.25 ± 0.68) and CGIIb (7.1 ± 0.73). Moreover, a* value obtained for EGX was significantly lower ($p < 0.05$) than those of EGIX (9.91 ± 0.91), CGIa (9.37 ± 0.19, and CGIIa (10.27 ± 0.31) as shown in Figure 8.

As for yellowness, chickens belonging to EGIX (8.95 ± 0.51), EGX (7.58 ± 0.7), EGXI (8.31 ± 0.56), CGIb (8.41 ± 0.51) and CGIIb (8.45 ± 0.42) showed significant differences when compared to EGIII (12.9 ± 0.24), EGIV (12.1 ± 0.08), EGV (12.41 ± 0.19), EGVI (12.84 ± 0.03), EGVII (13.62 ± 0.06), EGVIII (12.07 ± 0.06), CGIa (13.54 ± 0.22) and CGIIa (11.57 ± 0.65, Figure 9).

According to yellowness variation, samples frozen for 1 month significantly differed from each other ($p < 0.05$, Figure 10). Chickens of EGV showed a significantly higher ($p < 0.05$) b* value (13.95 ± 0.15) than EGVIII (10.93 ± 0.68), EGIX (10.87 ± 0.45), EGX (8.29 ± 0.55), EGXI (9.27 ± 0.59), CGIb (9.01 ± 0.34) and CGIIb (9.00 ± 0.44). Moreover, yellowness of EGX (8.29 ± 0.55), EGXI (9.27 ± 0.59), CGIb (9.01 ± 0.34) and CGIIb (9.00 ± 0.44) were significantly lower ($p < 0.05$) than b* of EGIII (12.7 ± 0/06), EGIV (12.58 ± 0.32), EGV (13.95 ± 0.15), EGVI (12.91 ± 1.11) and EGVII (12.88 ± 1.27).

One of the main criteria used to assess the quality of meat is the color of the flesh, a sensorial quality that highly influences the consumers' acceptance of meat (Listrat et al., 2016). Lightness value is particularly significant in white muscles and is connected to pH and drip loss. Lightness value is the primary factor influencing the color of poultry flesh, according to Barbut (1997). The ideal lightness range (L*) for poultry fillets is between 49 and 50. While fillets with values above that range are lighter in color and have low pH (pH 5.6), those with values below that range are darker and have high pH (pH > 5.9). In the current study, among color indicators, only a*(redness) was affected by herbs added to the diet; at 24 hours after cooling, it was lower than control (CGIIa) following the addition of peppermint, thyme rosemary and chamomile, and one month after freezing it was higher than control (CGIb and CGIIb) following the addition of onion powder to the diet. Earlier, Tashla et al. (2019) reported a non-significant difference in meat color (L*, a *, and b*) as a result of diet supplementation with garlic. On the other hand, Kirkpinar et al. (2014) found that diet supplementation using garlic oil did not affect the pH and yellowness of breast meat, but it significantly decreased its meat's lightness. Keokamnerd et al. (2008) reported non-significant changes in meat redness due to dietary supplementation with rosemary. Supplementation

of diets with thyme oil significantly affected redness values but did not affect the lightness and yellowness of breast fillets (Aksu et al., 2014). It was reported that the effects of supplementation of *Echinacea purpurea*, *Nigella sativa*, and their combined application on the meat color of broiler chickens are lower in L* than the control group (Nasir, 2009). Barbut (1997) found that L* (lightness) value was significantly higher in the cinnamon powder groups than in the control group, but there was no significant difference in a* (redness) and b* (yellowness) values among groups. Overall, the variation between L* and a* and b* values reflect the broad distribution of muscle pH values and myoglobin content in broiler breast meat, respectively (El Rammouz et al., 2004).

Drip loss measured 24 hours after cooling showed that chickens belonging to EGIV was significantly ($p < 0.05$) lower (1.6 ± 0.02%) than EGIII (3.17 ± 0.04%), EGV (3.24 ± 0.05%), EGVI (2.94 ± 0.12%), EGVII (3.4 ± 0.05%), CGIa (2.63 ± 0.07%) and CGIIa (3.16 ± 0.07%). EGVII whose chickens received chamomile as feed additive showed a significantly ($p < 0.05$) higher (3.4 ± 0.05%) yellowness than EGIV (1.6 ± 0.02%), EGVIII (2.28 ± 0.13%), EGIX (2.37 ± 0.3%), EGX (2.3 ± 0.47%), EGXI (1.8 ± 0.2%), CGIb (2.15 ± 0.17%) and CGIIb (2.1 ± 0.26%). The present result contradicted the findings of Begum et al. (2014) who postulated that drip loss did not differ among treatment groups fed with different types of herbs. According to Santos et al. (2004), who provided an illustration, fresh meat from slaughtered animals retains about 70% water, which is crucial to its quality but starts to leak away shortly after the animal dies. Additionally, boning and cutting may cause losses of 1% to 2%, and additional long-term storage may result in losses of up to 12%.

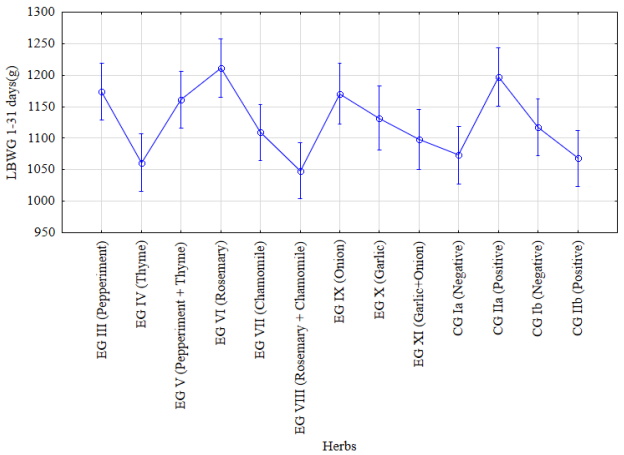

Figure 1. Variations in average live body weight gain (LBWG) during the whole experimental period (1-31 days of age of Ross 308 broiler chicken) among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

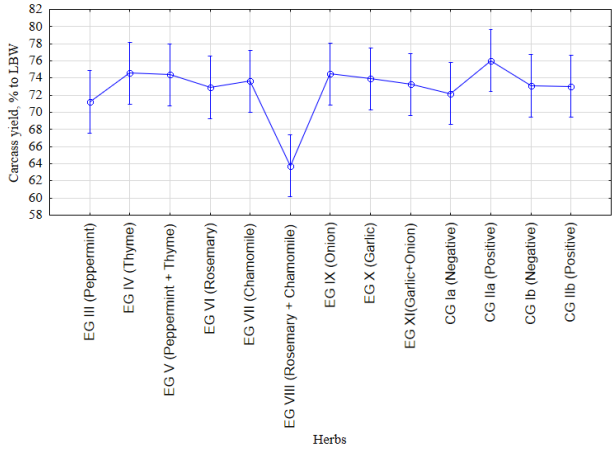

Figure 2. Variation of net weight percentage to live body weight (carcass yield) of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

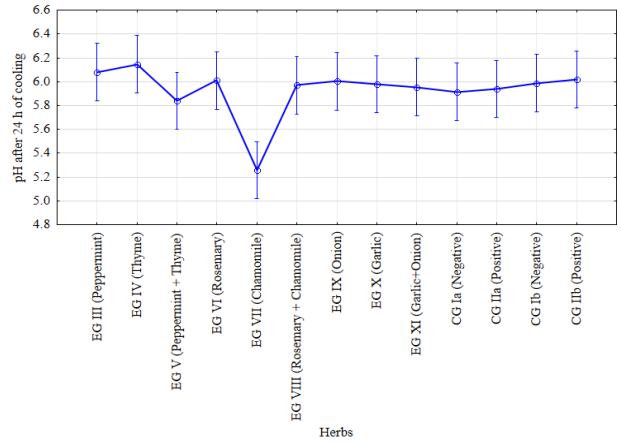

Figure 3. Breast meat pH variation after 24 hours of cooling of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

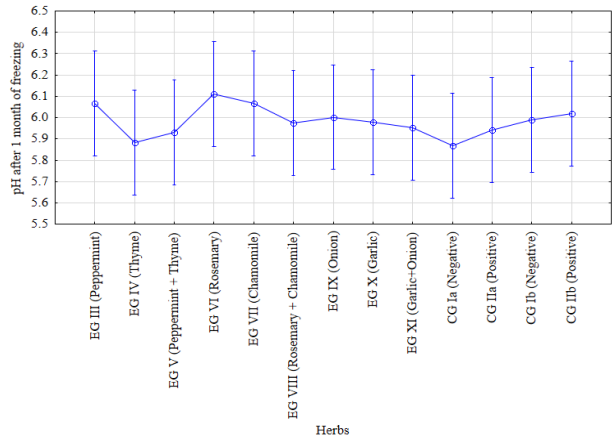

Figure 4. Breast meat pH variation after one month of freezing of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

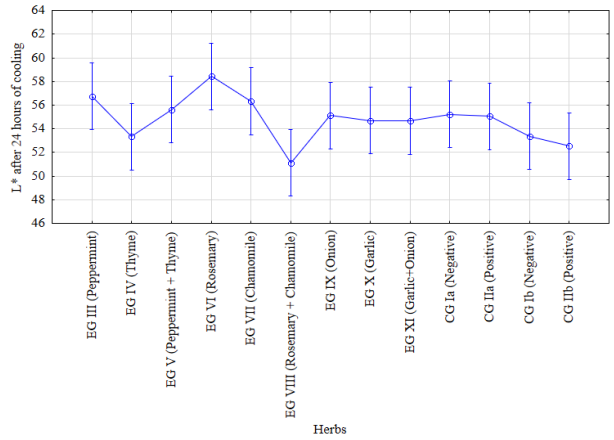

Figure 5. Breast meat lightness (L*) after 24 hours of cooling of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

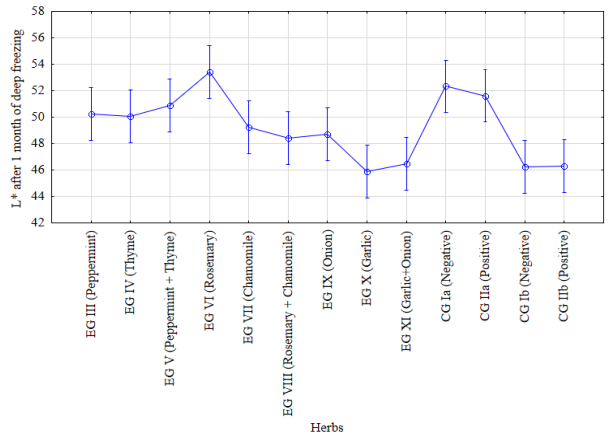

Figure 6. Breast meat lightness (L*) after one month of freezing of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

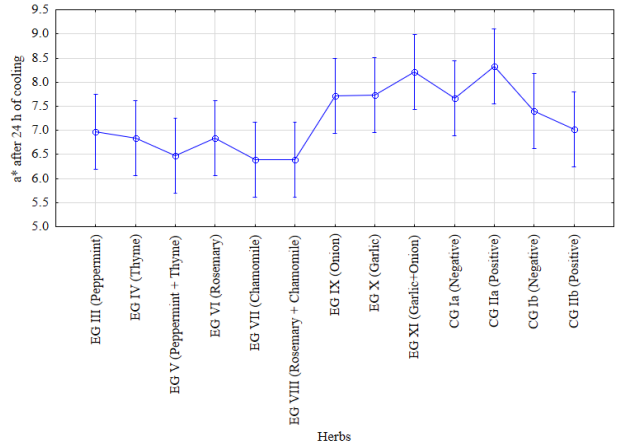

Figure 7. Breast meat redness (a*) after 24 hours of cooling of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

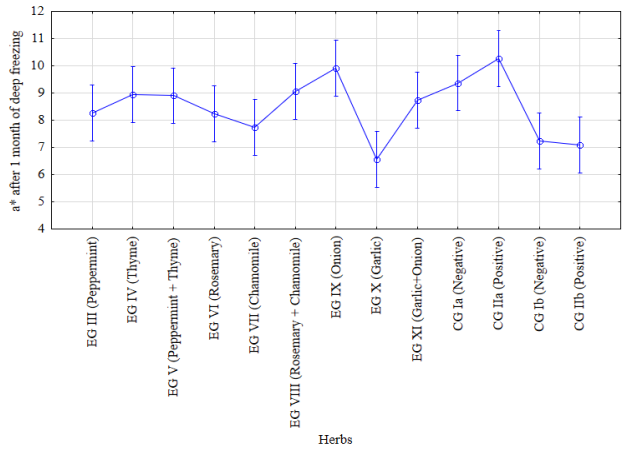

Figure 8. Breast meat redness (a*) after one month of freezing of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

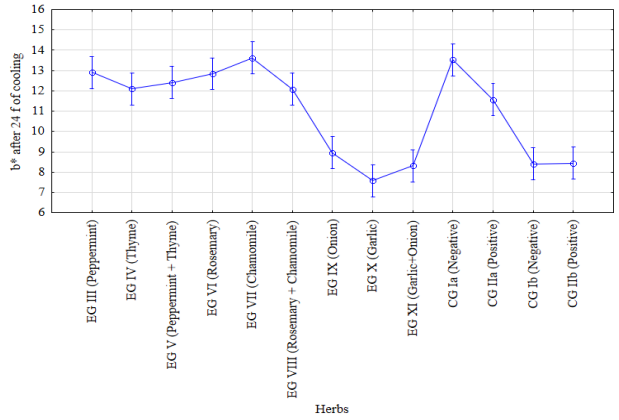

Figure 9. Breast meat yellowness (b*) after 24 hours of cooling of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

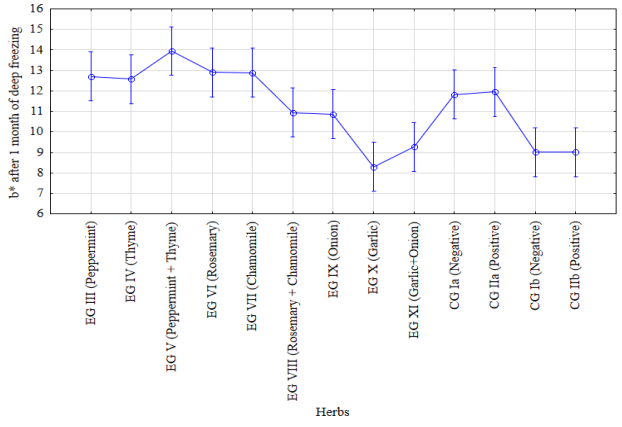

Figure 10. Breast meat yellowness (b*) after one month of freezing of Ross 308 broiler chicken among groups (EG: experimental groups, CGI: control groups of trial I, CGII: control groups of trial II)

CONCLUSION

Tested herbs had varying impacts on the overall chickens' performance. Their effects on physiology and meat quality were mostly comparable and sometimes better than that of antibiotics. In particular, adding rosemary to the diets could enhance chickens' body growth. Moreover, supplementing the diets with chamomile or a mixture of thyme and peppermint could increase the palatability of feed. Rosemary and thyme are more effective on feed conversion alone than in combination with other herbs. Although garlic (1%) and onion (1%) powders did not ameliorate feed conversion, they are well-known for enhancing chickens' immune systems. Overall, the use of the tested herbs as growth stimulants may reduce the need for antibiotics while offering farmers a practical answer. Future studies should be conducted on using the different herbs in other combinations and higher inclusion rates.

DECLARATION

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Authors' contribution

Roger Al Hanna conception of the idea, administration of the project, data collection and processing, and drafting and editing of the manuscript. The author checked and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The author declares no conflict of interest.

Ethical consideration

The author has made sure that the work complies with the journal's ethical issues for submission and publication.

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The Long-term Effects of Dietary Replacement of Fish Meal with Black Soldier Fly (*Hermetia illucens*) Larvae on Nutritional Content and Eggshell Quality in Layer Chickens

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ABSTRACT

Although the egg is one of the foods offering nutrients of high biological value, the diet of layer chickens can change these characteristics. The aim of this study was to evaluate the effect of a long-term dietary replacement of fish meal with maggot meal of black soldier fly larvae on egg quality of hens. A total of 480 one-day-old Isa brown chicks were randomly assigned to 4 dietary treatment groups. The groups were named T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal), and T3 (8% maggot meal). Each treatment group had 6 replicates of 20 chicks each. Data were collected on the eggshell quality parameters between 22 and 56 weeks of age. The results indicated that egg weight, shell weight, shape index, shell index, egg surface area, egg volume, density, yolk pH, albumen pH, yolk and albumen moisture content, yolk color, and yolk height were not influenced by the use of larval meals. Although the proportion of the yolk increased with age, there was no interaction between the use of fly larvae and the duration of its use for the collected parameters. However, the proportion of albumen, Haugh's unit in T1 and T3 treatments were higher than those of T0 and T2. The proportion of egg yolk, the yolk to albumen ratio, and the count of cracked eggs of T0 and T2 varied significantly compared to T1 and T3. Total egg fat decreased significantly as a result of the use of maggot meal. Total cholesterol, High-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol, and LDL/HDL ratio were lower in groups fed larvae meal, compared to the control group. It was concluded that the use of black soldier fly larvae meal during the entire rearing cycle and period of layers did not adversely affect the eggshell quality and nutritional content of the eggs.

Keywords: Black soldier fly, Cholesterol, Egg quality, Haugh unit, Larvae meal, Lipid

INTRODUCTION

The main characteristic of the egg among human foods is its richness in proteins and essential fatty acids (FAO, 2015). Feeding laying hens is crucial to optimize the excellent genetic potential of modern lines in terms of production performance and egg quality (Roberts, 2004; Leeson, 2011). Proteins and fatty acids are vital nutrients that can modulate not only the growth of hens but also the quality of the egg. The main concern of producers is the reduction of production costs through the high quality of the ingredients that ensure efficient production with the best possible profitability. In sub-Saharan Africa, particularly in Togo, the low availability in quantity and quality of feed combined with the high cost of animal proteins, such as fish meal, is one of the constraints to the growth of the poultry sector, which remains highly dependent on imports. Therefore, crucial to explore other unconventional and economically more profitable feed ingredients, available and not in competition with human food (Heuel et al., 2021). Also, in terms of quality, the fatty acid profile of the egg, whether in triglycerides or phospholipids, directly reflects the hen's food consumption of fatty acids (Chambers et al., 2017; Kralik et al., 2021). Additionally, the fat and cholesterol contents of meat are major characteristics of nutritional quality and health value due to the well-established relationship between diet, health, and well-being (Scollan et al., 2014). A reduced fat and cholesterol intake is associated with a reduced risk of chronic diseases, such as obesity, hypercholesterolemia, and cardiovascular disease (Chizzolini et al., 1999). According to Lessire (1995), chickens tend to incorporate dietary fatty acids, including long-chain monounsaturated fatty acids, into their tissues. This report implies that feed producers should use raw materials rich in essential fats to improve their nutritional values (Lessire, 1995).

There has been growing interest in using insects as a suitable source of protein in animal feed, and many studies have shown the impact of incorporating partially or totally defatted insect meals into poultry feed (Al-Qazzaz et al., 2016; Maurer et al., 2016; Marono et al., 2017). Although some studies have looked at substitution, they have only been short-lived and or focused on substituting a vegetal ingredient (soy) with an animal (insect) component (Kawasaki et al., 2019). Also, most of these studies have mainly identified only the protein or lipid potential of the maggots in the diet of laying hens on performance parameters (Al-Qazzaz et al., 2016; Maurer et al., 2016; Schiavone et al., 2017). However, few studies have shown interest in evaluating the effect of black soldier larvae meal, including in laying hen diet, on the quality values of egg, which will allow a better evaluation of this new feed ingredient in poultry production. One of the important concerns that remain with regard to the use of insect meal in the laying hen diet is whether the health of consumers will be affected by the extensive use of this ingredient. In this context, the aim of this study was to assess the impact of the long-term partial or total replacement of fish meal by undefeated maggot meal on the quality and safety of the layer eggs.

MATERIALS AND METHODS

Ethical approval

This study was carried out in strict compliance with the recommendations of the Guide for the Care and Use of Experimental Animals of the University of Lomé, Togo (008/2021/BC-BPA/FDS-UL). The protocol was approved by the Animal Experimentation Ethics Committee of the same University. All efforts were made to minimize chicken discomfort.

Animals and food

Maggot production

The meal of black soldier fly larva used in this study was produced at the Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, Lomé, Togo. *Hermetia illucens* larvae were produced by Sheppard et al. (2002) modified method. Freshly laid eggs on stacked wooden sticks were transferred to plastic dishes for incubation, where the larvae hatched between three and four days. The larvae were initially fed a diet of local brewers' grains. After a maximum development time of 6 days, the larvae were transferred to growth units on substrates composed of 1/3 brewers' grains and 2/3 palm kernel cakes. The larvae were fed these vegetarian by-products until day 14.

The larvae were harvested before they reached the pre-pupal stage to have a low degree of sclerotization of the cuticle and the resulting higher digestibility (Bosch et al., 2014). They were washed, followed by killing, and dried at 70°C for 48-72 hours, depending on the water content. Once dried, the maggot larvae were mechanically ground into a fine powder. The resulting product was a *Hermetia* larvae meal with 25% fat, 9.57% ash, and 41.1% crude protein (Table 1).

Table 1. Chemical-nutritional characteristics of the black soldier fly larvae meal and commercial fish meal

Chemical nutritional characteristics	Black soldier fly larvae	Fish meal 40%
Dry mater (%)	91.3	90.4
Crude protein (%)	41.1	40
Crude fat (%)	25	10
Cholesterol (% Ether extract)	79.42	22.10
Ash (%)	9.57	18.2
Crude fibre (%)	7	1.5
Lys (%)	4.90	1.91
Met (%)	1.84	1.54
Met+ Cys (%)	1.42	2
Tryptophan (%)	0.47	0.04
Threonine (%)	1.20	1.53
Calcium (%)	6.50	4
Total phosphor (%)	1.05	2.5
Metabolisable Energy (MJ/kg)	13	11

DM: Dry mater, EE: Ether extract, CF: Crude fibre, Lys: Lysine, Meth: Methionine, Cys: Cystine, Tryp: Tryptophan, Threo: Threonine, Ca: Calcium, P: Phosphore, ME: Metabolism energy, CNC: Chemical nutritional characteristics and AAS, Met+ Cys: Methionine + Cysteine

Animals

A total of 480 one-day-old Isa brown laying chicks were randomly assigned to four treatment groups; each treatment group had 6 replicates of 20 chicks each. The groups were named T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal), and T3 (8% maggot meal, Table 2). Feed was supplied in mash form in all four diets by CERSA feed unit (University of Lomé, Togo). The chicks were purchased from Incubel SA. (Hoogstraten, Belgium) and the experiment lasted 56 weeks (August 2020- September 2021). The chicks were reared on the floor on wood shavings in 24 cages of surface 4 m^2. The chickens were randomly allocated to an open hen house on deep litter with a natural environment, ventilation, temperature (21°C-27°C, and humidity (78%) to avoid position effects. The lighting program consisted of continuous light from weeks 1 to 8, followed by 18 hours of light from weeks 9 to 56.

Experimental device

The eggs used were collected from layer chickens in each group. The external and internal qualities of the eggs were determined at a 14-day interval throughout the experimental period. Parameters were collected between weeks 22 and 56 of age. The data collected on the two laying phases (between weeks 22 and 39, then weeks 40 and 56) made it possible to evaluate the interaction effects between the treatment and the age of the layers.

Table 2. Composition of experimental diet fed Isa brown laying chicks during the starter and grower and laying period

Treatments / Ingredients (kg/100kg)	Starter (1-8 weeks of age)				Grower (9-20 weeks of age)				Laying period (21-68 weeks of age)			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
White maize	52.8	51.8	51.6	51.1	55	54	54	54	54.5	54	54	53.9
Wheate bran	10	11	12	12.5	15	16	16.5	16.5	9.5	10.3	10.9	11
Fish meal	8	4	2	0	8	4	2	0	8	4	2	0
Soya seed	20	20	19	19	13	13	12.5	12	14.5	14	13.3	12.8
Oyster shell	2	2	2	2	5	5	5	5	8	8	8	8
Concentrate	7	7	7	7	4	4	4	4.5	5	5	5	5.5
Lysine	0.1	0.1	0.2	0.2	0	0	0	0	0.2	0.3	0.4	0.4
Methionine	0.1	0.1	0.2	0.2	0	0	0	0	0.3	0.4	0.4	0.4
Maggot meal	0	4	6	8	0	4	6	8	0	4	6	8
Total (Kg)	100	100	100	100	100	100	100	100	100	100	100	100
Chemical nutritional characteristics												
DM[1] (%)	98.68	98.55	98.42	98.35	98.12	97.89	97.82	97.82	98.75	98.64	98.56	98.55
CP (%)	20.26	20.37	20.25	20.30	17.12	17.23	17.11	17.07	18.29	18.35	18.25	18.29
EE (%)	7.34	7.94	8.06	8.36	6.15	6.75	6.96	7.16	6.38	6.89	7.06	7.27
Ash (%)	1.56	1.62	1.62	1.65	1.49	1.55	1.55	1.52	1.26	1.28	1.28	1.26
CF (%)	4.63	4.90	5.04	5.18	4.82	5.10	5.23	5.31	4.49	4.59	4.72	4.80
Lys (%)	1.22	1.33	1.45	1.50	0.95	1.07	1.11	1.17	1.15	1.33	1.45	1.51
Meth (%)	0.63	0.65	0.76	0.77	0.43	0.45	0.46	0.49	0.72	0.84	0.85	0.87
AAS (%)	0.86	0.80	0.80	0.88	0.62	0.60	0.58	0.58	0.93	1.00	0.98	0.98
Tryp (%)	0.11	0.11	0.11	0.11	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08
Threo (%)	0.33	0.33	0.33	0.33	0.26	0.26	0.26	0.25	0.25	0.25	0.24	0.23
Ca, (%)	1.71	1.81	1.86	1.91	2.29	2.39	2.44	2.53	2.92	3.02	3.07	3.13
Total P, (%)	0.58	0.53	0.51	0.47	0.60	0.55	0.53	0.50	0.67	0.62	0.59	0.57
ME (MJ/kg)	12.1	12.1	12.1	12.1	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5

EE: Ether extract, CF: Crude fibre, Lys: Lysine, Meth: Methionine, Cys: Cystine, Tryp: Tryptophan, Threo: Threonine, Ca: Calcium, P: Phosphore, ME: Metabolism energy, AAS: methionine + cysteine. NB: Chemical nutritional characteristics are calculated based on the feed

External egg quality

Six eggs per replicate (48 eggs per treatment) were collected every 4 weeks. The egg weight (MEW) per treatment was calculated according to the formula MEW = ∑W/N. Where W is egg mass and N denotes the number of eggs. The width and height of each egg were measured (Breadth-B; Length-L) with a digital caliper with an accuracy of 0.01 mm. The following parameters were calculated using the weight of the eggs.

Shape index = B/L (Panda, 1996), Shell index (SI g/cm2) = (Shell weight / Shell surface area, Rodriguez-navarro et al., 2002), and the volume V (cm^3) is $\pi/6 \times L$ (cm) $\times B^2$ (cm^2, Bonnet and Mongin, 1965). Moreover, the density was calculated as density (g/cm^3) = MEW/V, where MEW(g) is egg weight and V (cm^3) is the volume of the egg. The acoustic test was repeated for all eggs using the same device (Digital caliper blet, China). Each egg was classified as intact or cracked.

Internal egg quality

The weight of the yolk and that of the albumen were measured using an electronic balance with a sensitivity of 0.01 g. Albumen height and Haugh unit (HU) were measured using an electric micrometric (Futura, Lohne, Germany) tripod with an accuracy of 0.01 micrometer. Egg yolk color was measured using the Roche color scale (RCF), which is an industrial color scale with visual ratings ranging from 1 (light yellow) to 16 (dark orange). The pH measurement (pH digital meter OAKTON pH700) was taken individually for each egg in albumen and egg yolk by immersing the probe inside the sample solution. Between two measurements, the electrode was cleaned with distilled water and then recalibrated using a buffer solution (Silversides and Budgell, 2004).

Yolk total fat

At 38-41weeks of age, lipid and cholesterol content were measured. The materials used for the extraction of fat were the Soxhlet and the oven. The reagent used for this analysis was hexane. The test portion (M_1) of the sample was about 0.5 g to 1 mg and the time of analysis was about 1 hour. The samples were weighed and placed in cellulose cartridges. These cartridges were inserted into crucibles containing 30 ml of hexane previously placed under vacuum mass, M_2, in the oven for 10 minutes and cooled in a desiccator. The crucibles containing the hexane and the cartridges containing the samples were placed in the Soxhlet. The fat extraction took place in three stages for one hour: boiling, rinsing, and hexane recovery. The crucibles were then recovered, kept in an oven for 15

minutes, cooled in a desiccator, and weighed (M_3.) The fat content was obtained using the following formula:

$$\text{Fat contain} = \frac{M_3\text{-}M_2}{M_1} \times 100$$

Cholesterol profiling

Total cholesterol and High-density lipoprotein (HDL) cholesterol were determined using the respective cholesterol assay kit (Fortress Diagnostics, UK). The kit contained the standards, assay reagent, and color development reagent (Pasin et al., 1998).

Total cholesterol

The lipid extract obtained was used for the determination of the cholesterol profile. Therefore, 10 µl of the sample/standard and 1000 µl of cholesterol reagent were added. The solution was vortexed and incubated for 5 minutes at 37°C. The absorbance of the color developed by the sample or standard was measured against the reagent blank at 505 nm.

$$\text{Concentration of Cholesterol} = \frac{\Delta \text{ absorbance } of\ sample}{\Delta \text{ absorbance } of\ Standard} \times standard\ concentration.$$

HDL and LDL

A portion of 0.4 ml of the precipitation reagent was piped into a centrifuge tube. 0.2 ml of sample was taken, mixed, and allowed to stand at room temperature (25°C) for 5 minutes. The solution was centrifuged at 4000 rpm for 10 minutes. 100 µl of the sample/standard and 1ml of cholesterol reagent were added. The solution was vortexed and incubated for 5 minutes at 37°C. The absorbance of the developed color of the sample or standard against the reagent blank was then measured (Pasin et al., 1998).

$$\text{Concentration HDL} = \frac{\Delta \text{ absorbance of sample}}{\Delta \text{ absorbance of Standard}} \text{ x standard concentration}$$

Statistical analysis

All external and internal egg quality data were analyzed using graph pad prism 9.0 software. One-way analysis (ANOVA one-way) was used for data analysis. The comparison of the means was made using the Tukey test with $p < 0.05$ as the significance threshold. For the assessment of the interaction between age and treatment, a two-way factorial analysis of variance (ANOVA two-way) was used. Principal Component Analysis (PCA) was performed using XLSTAT software (version 2021) to assess the correlation between parameters. The results are presented as mean ± standard error of the mean.

RESULTS

Larvae meal and external egg quality

The physical parameters of the collected eggs showed no significant difference among the treatment groups; Egg weights, shell weight, shape index, shell index, egg surface, egg volume, and density were not influenced by the use of larva meal ($p > 0.05$). However, cracked eggs in the control group were significantly higher ($p < 0.05$) than those in the treatment which received the maggot meal (Table 3).

Larvae meal and internal eggs quality

The results showed a significant influence on the percentage of albumen, the albumen height, and the Haugh unit of the treatments T1, T2, and T3, which were greater than those of the control group ($p < 0.05$). The proportion of egg yolk and the ratio between the yolk and the albumen of the control hens were statistically higher. However, yolk pH, albumen pH, yolk and albumen moisture content (Mc), and yolk color and height were not affected by the treatments ($p > 0.05$, Table 4).

Egg quality parameters of the laying phases

Egg weight

The egg weights of the second phase of laying were greater than the weight of the first phase (Table 5). There was no interaction between maggot meal feeding and hen age on egg weights. The proportion of the egg albumen in the second phase of laying was statistically lower than the proportion of the first phase ($p < 0.05$). There was no interaction between maggot diet and hen age on the percentage of albumen ($p > 0.05$). The proportion of yolk in the second phase of laying was Significantly higher than the proportion of the first phase ($p < 0.05$). The treatments showed no interaction between maggot meal feeding and hen age on egg yolk percentage ($p > 0.05$). The Haugh unit of the egg albumen of the second phase of laying was significantly lower than those of the first phase ($p < 0.05$). The treatments showed no interaction between maggot meal feeding and the age of hens in the Haugh unit ($p > 0.05$).

Total lipid level of egg yolk

The fat content of eggs in the control group was significantly higher than that of hens fed maggot meal ($p < 0.05$, Figure 1). This rate was statistically similar for the yolk of T1 and T3 eggs. Hens received 75% maggot meal showed yellows with a low proportion of fat (Table 4).

Total cholesterol level of egg yolk

The total yolk cholesterol level of the control group hens fed fish meal was significantly higher than T1 hens ($p < 0.05$, Figure 2). This level was higher in treatment T0 (445.6 mg/100 g of egg yolk), compared to 340, 393.8, and 410.7 mg/100g for T1, T2, and T3), respectively. The proportion of cholesterol in the yolk increased significantly with the increase in the level of larvae meal in the feed ($p < 0.05$) however remained statistically similar for the yolk of T2 and T3 eggs ($p > 0.05$).

High-density lipoprotein of egg yolk

The egg yolk HDL cholesterol level of the control group hens fed fish meal was significantly higher than maggot meal ($p < 0.05$, Figure 3). The proportion of HDL cholesterol in yolk increased with the increase in the level of maggot meal in the feed. It was significantly higher for the yolk of T2 and T3 eggs, compared to T1 ($p < 0.0001$). This rate was very high for batch T0 (349.4 mg/100 g of

egg yolk against 280.3, 331.6, and 330.3 mg/100g for T1, T2, and T3, respectively).

Low-density lipoprotein of egg yolk

The yolk low-density lipoprotein (LDL) cholesterol level of the control group hens fed fish meal was significantly higher than maggot meal ($p < 0.05$). The proportion of LDL cholesterol in yolk increased with the increase in the level of maggot meal in the feed. It was significantly higher for egg yolk T3 compared to lower T1 and T2 and statistically comparable ($p < 0.05$, Figure 4). This rate was very high for T0 treatment (96.19 mg/100 g of egg yolk against 59.73, 62.24, and 98.2 mg/100 g for T1, T2, and T3, respectively).

Atherogenicity index

The LDL/HDL ratio appeared to increase with the incorporation rate of maggot meal. It was significantly low compared to the control for 4% and 6% incorporation; however statistically comparable between 8% larvae meal inclusion and the control group ($p < 0.05$, Figure 5).

Principal component analysis

Correlation circle

Figure 6 shows the degree of connection between the variables. Yolk height, yolk color, albumen percentage, and Haugh unit were positively correlated. Similarly, total fat content, total cholesterol level , and HDL level on the one hand, then shell area, albumen moisture content, volume, and egg weight on the other were closely related and positively correlated. The shell area and albumen moisture content were negatively correlated with albumen pH ($r = -0.97$ and $r = -0.99$, respectively).

Treatment

The use of soldier fly larvae meal showed two different groups of characteristics. The eggs of groups T1, T2 and T3 from hens fed with soldier fly larvae meal had comparable characteristics. The eggs of the control groups (T0) showed different characteristics (Figure 7).

Combination of correlation circle and treatment

Combining the correlation circle with the treatment map showed that T1 and T3 were grouped by moisture content, albumen height, egg weight, shell area, volume, and Haugh unit on axis 1 (Figure 8). However, on axis 2, the T3 treatment was characterized as the T0 eggs by the high values of LDL level and egg density. The T0 treatment was distinguished from T3 by the yolk percentage, the total cholesterol level, and the yolk/albumen ratio.

Table 3. Effect of larvae meal on the external quality of the egg (mean values)

Parameters \ Treatments	T0	T1	T2	T3	P-Value
Egg weight (g)	53.01	53.34	53.11	54.87	0.2284
Shell weight (g)	7.06	7.08	7.04	7.35	01744
Shape index	0.76 ± 0.003	0.77 ± 0.003	0.76 ± 0.002	0.77 ± 0.003	0.3753
Shell index	0.087 ± 0.001	0.087 ± 0.001	0.087 ± 0.001	0.088 ± 0.001	0.5323
Shell proportion (%)	11.98	11.91	12.23	12.07	0.6381
Cracked eggs (%)	0.44	0.16	0.17	0.05	0.0186
Egg area (Cm^2)	80.60 ± 0.67	81.03 ± 0.60	80.83 ± 0.80	82.22 ± 0.74	0.3729
Volume (Cm^3)	48.81 ± 0.52	49.06 ± 0.46	49.36 ± 0,65	50.47 ± 0.63	0.1837
Density	1.08 ± 0,01	1.07 ± 0.01	1.08 ± 0.01	1.09 ± 0.01	0.8541

[a,b,c] The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0: 8% fish meal, T1: 4% maggot meal and 4% fish meal, T2: 6% maggot meal and 2% fish meal, and T3: 8% maggot meal

Table 4. Effect of larvae meal on the internal composition of the egg (mean values)

Parameters \ Treatments	T0	T1	T2	T3	P-value
Albumen (%)	60.95	62.75	62.32	62.35	0.0045
Albumen height (mm)	6.79 ± 0.06^b	7.34 ± 0.07^a	6.88 ± 0.06^b	7.28 ± 0.86^a	0.0001
Albumen moisture content (%)	83.10	83.40	83.21	83.91	0.3659
Yolk (%)	25.01	24.04	24.14	24.14	0.0071
Yolk height (mm)	14.11 ± 0.10^b	14.49 ± 0.08^a	14.22 ± 0.09^b	14.30 ± 0.11^b	0.0431
Yolk index	0.44 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.3080
Color of yolk	7.75 ± 0.27	8.03 ± 0.39	8.13 ± 0.39	8.04 ± 0.30	0.8830
Yolk moisture content (%)	60.92	58.95	57.34	58.82	0.1652
Yolk/albumen	0.41 ± 0.00^a	0.39 ± 0.01^b	0.40 ± 0.01^a	0.40 ± 0.01^a	0.0003
Haugh unit	84.18 ± 0.84^b	87.61 ± 0.83^a	85.71 ± 0.83^b	87.69 ± 1.1^a	0.0008
Albumen pH	7.76 ± 0.01	7.73 ± 0.01	7.75 ± 0.01	7.70 ± 0.02	0.0623
Yolk pH	5.34 ± 0.04	5.35 ± 0.06	5.34 ± 0.07	5.33 ± 0.02	0.9195

[a,b,c] The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0: 8% fish meal, T1: 4% maggot meal and 4% fish meal, T2: 6% maggot meal and 2% fish meal and T3: 8% maggot meal, Mc: Moisture content.

Table 5. Effect of maggot meal and age of layers on egg weight, shell proportion, albumen proportion, Haugh unit, and yolk/albumen ratio (mean values)

Parameters	Laying period								P-value		
	22-39 weeks				40-56 weeks						
	T0	T1	T2	T3	T0	T1	T2	T3	Diet	Age	Diet×age
Egg weight (g)	50.22±4.58^{b}	51.42±4.1^{b}	49.97±5.20^{b}	51.42±4.86^{b}	56.36±3.97^{a}	55.75±3.83^{a}	57.11±4.59^{a}	58.58±4.99^{a}	0.1088	<0.0001	0.2145
Shell (%)	12.40	12.20	13.00	12.39	11.58	11.63	11.47	11.76	0.4762	<0.0001	0.0987
Albumen (%)	62.38	64.25	62.18	64.11	61.03	61.24	60.25	61.01	0.0003	<0.0001	0.0839
Haugh unit	85.36±3.33^{b}	88.97±3.64^{a}	85.50±3.34^{b}	88.09±3.97^{a}	83.01±3.53^{c}	85.81±3.42^{d}	83.79±3.91^{c}	85.05±3.20^{d}	<0.0001	<0.0001	0.4620
Yolk (%)	24.56	23.44	23.67	23.50	25.45	24.64	24.61	24.68	0.0004	<0.0001	0.9096
Yolk/Albumen	0.39±0.04^{c}	0.36±0.04^{d}	0.38±0.04^{c}	0.36±0.04^{d}	0.42±0.04^{a}	0.40±0.05^{b}	0.41±0.02^{a}	0.40±0.04^{b}	0.0007	<0.0001	0.6386

^{a,b,c,d,e} The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0: 8% fish meal, T1: 4% maggot meal and 4% fish meal, T2: 6% maggot meal and 2% fish meal, and T3: 8% maggot meal.

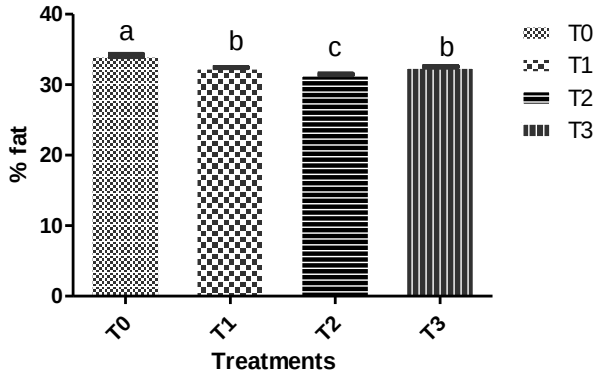

Figure 1. Effect of different level of larvae meal on the variation of the total fat content of the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

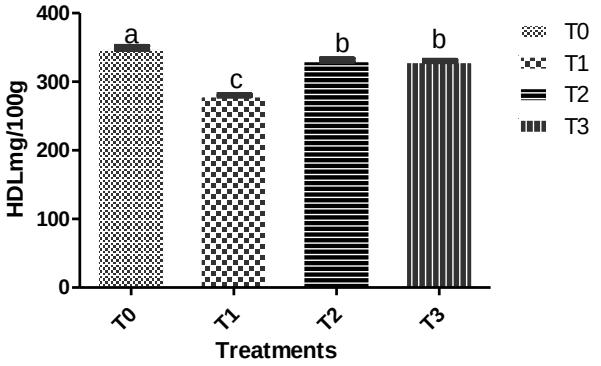

Figure 3. Effect of different level of larvae meal on the variation of the HDL cholesterol level of the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

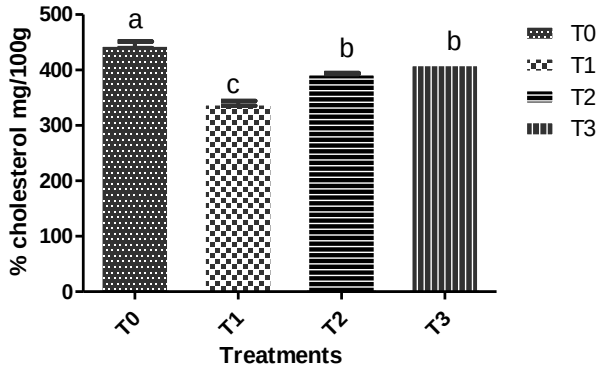

Figure 2. Effect of different level of larvae meal on the adequacy of total cholesterol levels in the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

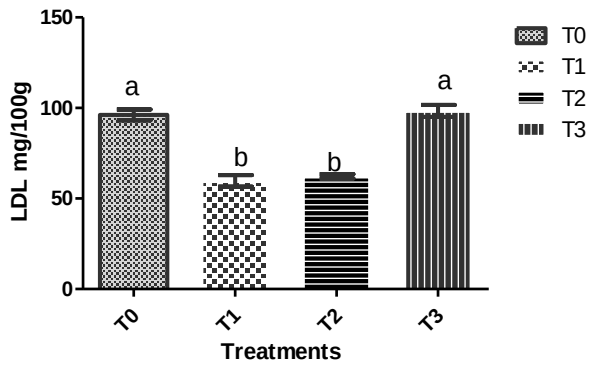

Figure 4. Effect of different level of maggot meal on the level of HDL cholesterol levels in the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

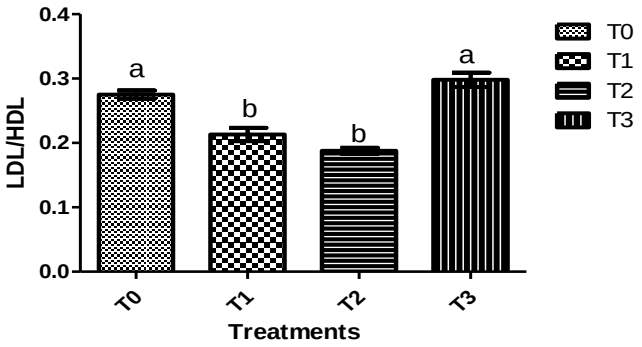

Figure 5. Effect of different level of maggot meal on the level of HDL cholesterol levels in the Isa brown laying chick's eggs. [a,b,c] The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

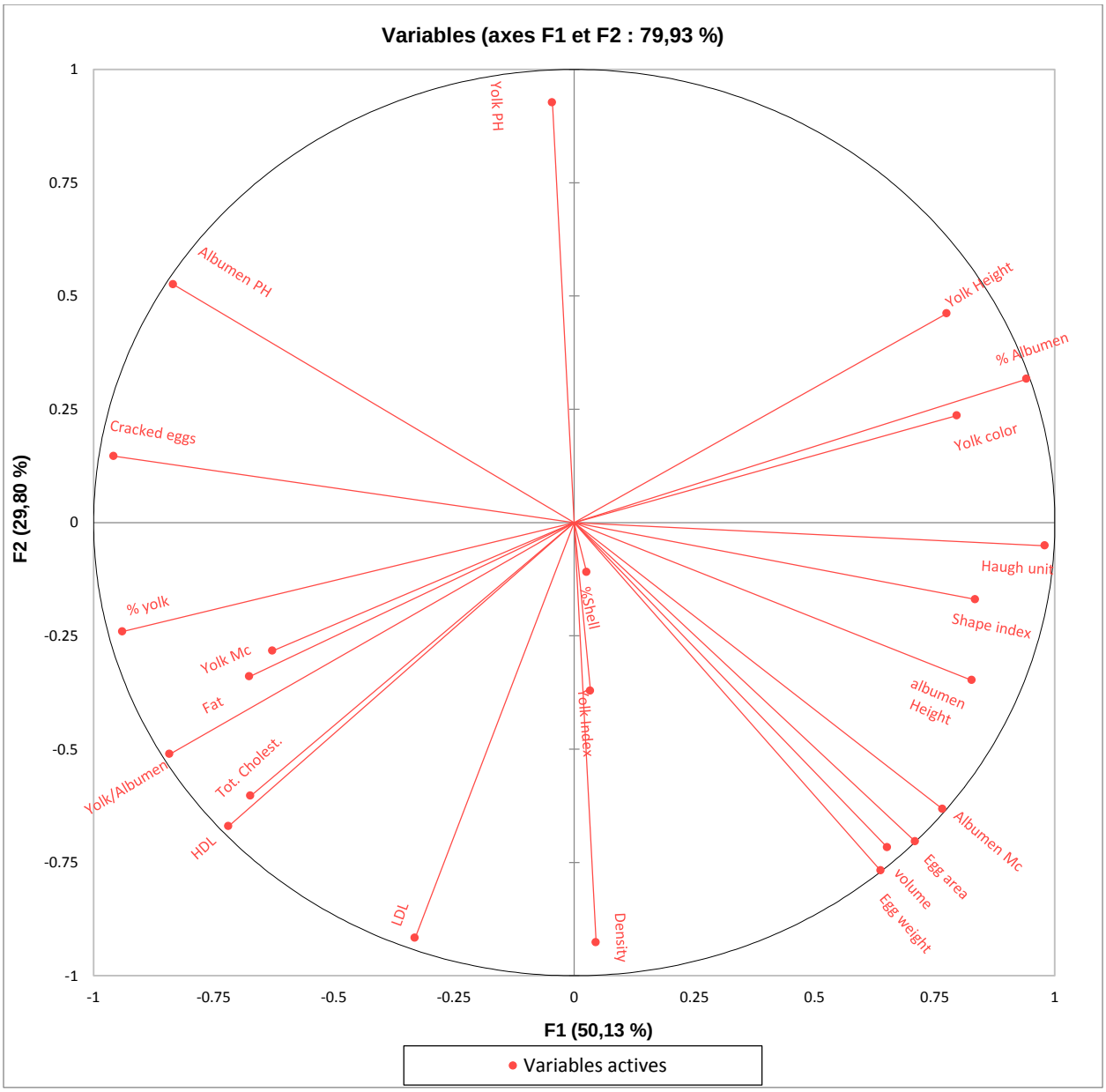

Figure 6. Correlations between measured parameters. Haugh unit, Shape index, Volume, Albumen height, Albumen moisture content, Egg surface area, Egg weight, Egg shell proportion (%), Yolk index; Density, Total cholesterol, HDL, LDL, Yolk/albumen ratio, Yolk proportion (%), Cracked eggs, Albumen pH, Yolk pH, Yolk height, Yolk color, Albumen proportion (%).

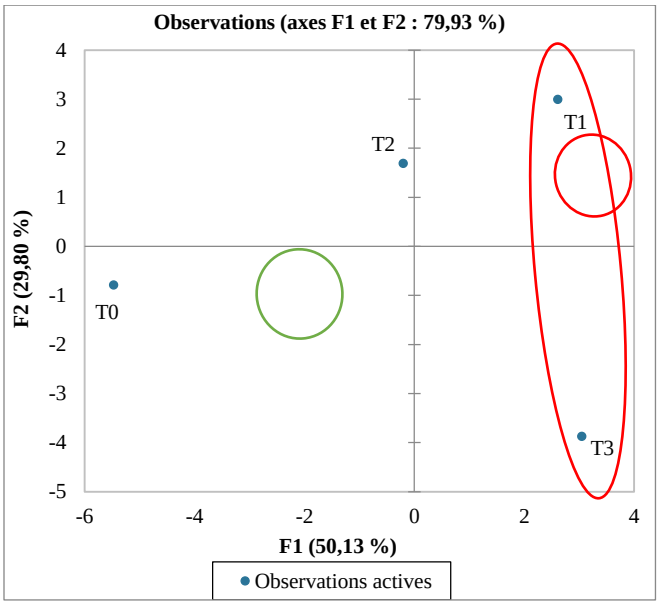

Figure 7. The differences and similarities of the treatments

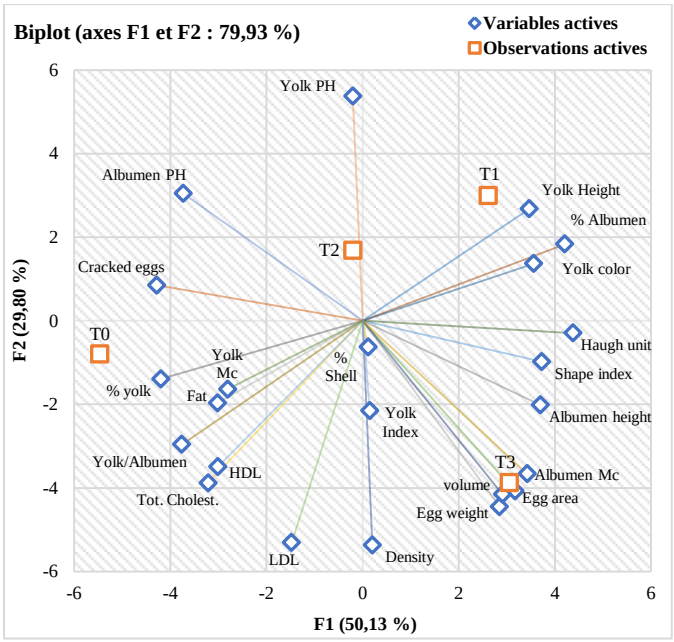

Figure 8. Combination of correlation circle and similarities of the treatments

DISCUSSION

In this study, the mean value of the Haugh units was between 84.18 and 89.69 (Table 4), which is similar to the values observed by Akpodiete et al. (1998), indicating that fresh eggs had an HU value of more than 72. Although age and storage time are the main factors influencing egg freshness, the source of dietary protein and its amino acid profile can also modulate the albumen height and, thus, the Haugh unit of the egg. Therefore, the improved Haugh unit of eggs from hens fed soldier fly larvae meal is explained by the higher lysine content (Roberts, 2004). The significant values of albumin height and Haugh's unit are in agreement with the results observed by Brah et al. (2017) and Park et al. (2017) using grasshopper meal and black soldier fly pupa meal, respectively, in laying hen feed. Present results showed that the albumen is the part of the egg most affected by the larvae meal (Akpodiete et al., 1998; Irawan et al., 2019).

The yolk/albumen ratio was higher in control eggs receiving 8% fish meal. The increase in the proportion of yolk in the eggs of hens fed with the control feed (T0) was due to the reduction in the proportion of albumen. Larvae meal had no effect on the yolk color in the present study. This result is consistent with those found in previous studies (Agunbiade et al., 2007; Amao et al., 2010). Dietary treatment did not affect egg and shell weight ($p > 0.05$). The results of this study are consistent with the findings of Akpodiete et al. (1998), who showed that the use of soldier fly larvae meal does not influence egg weight and yolk color ($p > 0.05$). Similarly, the results of this study confirmed the deduction of Olteanu et al. (2012), who concluded that feeding insect meals has no significant effect on egg weight. The yolk color of hens fed black soldier fly larvae meal showed no significant difference from the yolk of the control group.

Although the larvae used were raised on plant substrates, this result indicates that there was no possible transfer of carotenoids or xanthophyll pigments from these plant residues by the larvae into the coloration of the yolk (Suparman et al., 2020). The average value of the egg shape index and egg surface area for hens fed the larvae meal were 0.76-0.77 and 80.83-82.22 cm^2, respectively. These values were not significantly different from the control group ($p > 0.05$). Similar results were also observed by the report of Agunbiade et al. (2007) who showed that the egg shape index was not affected significantly with the use of maggot meal in a layer diet. The egg shape index determines the physical quality of the egg and provides information on the functional state of the isthmus and its diameter which it is closely dependent. The results of the present study indicated that larvae meal had no negative effect on shell gland muscle tone. Similarly, egg density, egg volume, albumen pH, and yolk pH were not influenced by larvae meal. However, the cracked egg content of the T1 and T3 groups was relatively low. Reducing these non-integrity defects of the shell that promote the penetration of pathogenic bacteria into the egg would reduce the risk of food poisoning linked to the consumption of eggs from this production.

In the current study, the increase in egg weight, yolk proportion, and decrease in Haugh unit during the second phase of egg laying would be due to the effect of age (Table 5). Indeed, as the hens' age increases, lipid catabolism increases due to a greater secretion of bile. This allows for better yolk formation. The improvement in yolk proportion is consistent with the results of Bejaei and

Cheng (2020). This study also showed that the large variations in the results obtained in several previous studies with the use of fly larvae for the assessment of egg parameters would depend on the age or laying phase of the layers used for the experiment concerned. In this study, the age of the hen and the level of fly larvae meal incorporated into the feed had effects on the proportion of albumin and the Haugh unit, but statistical analysis showed no interaction between these two factors. This observation is contrary to the results of Bejaei and Cheng (2020), who found no improvement in albumin weight and no significant effect on the Haugh unit. This contradiction would be due to the very high level of larvae meal incorporated and their method of analysis. In a recent study, Star et al. (2020) found no significant effect on the Haugh unit with the inclusion of soldier fly larvae in the hen's diet but found the period of their experiment too short. The same authors supported their results by reporting the age and laying phase of their laying hens. The results of the current study are in agreement with Akpodiete et al. (1998), who conducted a 56-day experiment to determine the effect of maggot meal supplementation in chicken diets and found that albumin weight was significantly affected. This observation was different from those of Shah (2020), who explained their result by the difference in age of the laying hens.

The chemical composition of the eggs obtained in this study is in agreement with those of Shin et al. (2013), who observed depletion of the lipid content of the egg yolk of hens fed with black soldier fly larva meal. Total lipid levels decreased with the replacement of fish meal with larvae meal (Figure 1). Eggs from hens fed larvae meal had significantly lower fat levels than control eggs ($p < 0.05$). According to Hossain and Blair (2007), chitin causes a decrease in triglyceride levels, which is the main component of total lipids. These authors showed a reduction in serum cholesterol and triglycerides by incorporating commercial chitin in the diet of broilers from 1 to 21 days of age at zero, 25, 50, and 75 g/kg. The effect of chitin on triglyceride reduction could be attributed to its positive charge capable of attracting negatively charged bile acids and free fatty acids during digestion (Prajapati and Patel, 2010). The lipid depletion was 1.6 g less fat in 100 g of egg yolk from hens receiving only larvae meal (T3) compared to the control. These results are also consistent with the results of Secci et al. (2018), who showed a decrease in yolk lipid levels with the use of maggot meal. Eggs from layers fed with larvae meal have reduced fat content, which could be advantageous for consumers who increasingly desire less fat in their food for their health and safety. Cholesterol levels in the yolk are known to be more regulated by endogenous metabolism than by dietary cholesterol. The average cholesterol level obtained during the current test was 445.6 mg/100g for T0. This was between 340 mg/100 g and 410, 7 mg/100 g or 3.4 to 4.1 mg/g of egg yolk for the eggs of layers fed larvae meal (Figure 2). The mean egg yolk cholesterol level in this study was lower than the values obtained by Irawan et al. (2019) and Cayan and Erener (2015), who found cholesterol levels of 5.20-5.90 mg/g and 8.34-9.24 mg/g for egg yolk, respectively.

Fat and cholesterol contents were positively correlated with the egg yolk proportion ($r = 0.79$, Figure 5). Cholesterol level in the egg yolk depends mainly on the balance between cholesterol biosynthesis and excretion (Griffin, 1992). The reduction in cholesterol levels in eggs in groups fed larvae meal is thought to be due to the fatty acid profile of larvae meal (Mazalli et al., 2004). Previous research testing meal from black soldier fly larvae as a feed ingredient in laying hens (100% replacement of soybean meal), observed an 11.7% reduction in yolk cholesterol content in hens receiving the experimental diet comprising the black soldier fly larvae meal compared to a control group fed soybean meal (Secci et al., 2018). This study shows that black soldier fly larvae meal resulted in a decrease in the cholesterol level of the eggs. As well as the triglycerides, this reduction in cholesterol level would also depend on chitin, a natural component of the insect exoskeleton, which exerts an attraction on negatively charged bile acids and free fatty acids (Prajapati and Patel, 2010). The reduction observed was 105mg/100g, 51.8 mg/100g, and 35mg/100g for cholesterol in egg yolk T1, T2, and T3, respectively (Shin et al., 2013). Cholesterol and its esters are found only in egg yolk, where they form an emulsion of LDL and HDL. HDL and LDL cholesterol positively correlated with total cholesterol ($r = 0.97$ and $r = 0.81$, respectively), increased with the black soldier fly larvae meal use rate. This study showed that larvae meal would have induced an important secretion of vitellogenin, which oocytes represent the principal target of accumulation (Voet and Voetová, 1995). Indeed, egg yolk lipoproteins known as vitellogenin have biochemical qualities similar to mammalian serum lipoproteins (Voet and Voetová, 1995). They are synthesized in the liver and secreted into the blood, where they are captured by oocytes. The atherogenic index (LDL/HDL) is a relevant factor in assessing the nutritional quality of the egg for consumer protection. Eggs with a low atherogenic index are good for delaying atherosclerosis and, therefore, the risk of cardiovascular disorders (EL-Wakf et al., 2010).

CONCLUSION

Black soldier flies maggot meal had no negative influence on the eggs' external, internal, and nutritious quality. It improved the proportion of albumen, a valuable source of high-quality protein. Egg freshness and chemical quality of 4% and 8% incorporation eggs have been improved. Meal from soldier fly larvae can reduce egg yolk fat and cholesterol content. In addition, larvae meal can be used at an incorporation rate of 4% or 8% in the feed throughout the rearing cycle of layers. However, dietary cholesterol that increases the sensitivity of LDL to oxidation and potentiates the adverse effects of dietary saturated fats in

humans remains very high in the egg. Following dietary recommendations that indicate the need to limit cholesterol intake to less than 300 mg/day, it would be desirable to consume eggs in moderation. Based on the results of the current study, the authors conclude that the long-term inclusion of black soldier fly larvae meal in laying hen diets is safe and feasible.

DECLARATIONS

Acknowledgments

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Authors' contribution

Kodjo Gnatépé Mlaga designed, performed the experiment (project administration Methodology; analysis), and wrote the manuscript. Komi Attivi participated in the manipulations and reading of the manuscript; Komi Agboka and Kokou Tona participated in the funding acquisition and reading of the manuscript. Elolo Osseyi supervised, analyzed data, and approved the final manuscript. All authors read and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The authors declare no conflict of interest.

Ethical consideration

The authors have verified the ethical issues related to plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy).

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Effect of Selenium-based Diets on Zootechnical Performance, Hematological Parameters, and Relative Weight of Internal Organs in Broiler Chickens

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ABSTRACT

Two sources of selenium are commonly used in poultry nutrition, the organic and the inorganic forms. This study was carried out to investigate the comparative effect of Sasso broiler breeder feed supplemented with sodium selenite (SS) and selenomethionine (SM) on the zootechnical performance, hematology, and hatching process of chickens. A total of 120 female broiler breeders and 12 roosters of Sasso strain at 47 weeks were equally assigned to three treatments with four replicates per each, including 10 breeders crossed with 1 rooster. The treatment groups were broiler breeders fed a basal diet without selenium supplementation (control), chickens fed the basal diet supplemented with SS, and breeders fed the basal diet supplemented with SM. The inclusion level of each selenium was 0.2 ppm. The collected data included feed intake and egg weight during 8 weeks. In the end, blood samples were collected for hematological investigations. A total of 150 hatching eggs were collected from each treatment. After recording their weight, the eggs were incubated at adequate temperature and relative humidity. On day 18 of incubation, the eggs were weight again, candled, and transferred into the hatcher. Each egg was individually checked every 3 hours during the last 3 days of incubation for hatching events determination. The results showed that breeders fed SM had the lowest feed conversion ratio. There was an increase in the majority of blood parameters in breeders fed SM, compared to other treatments. The lowest duration of the hatching events was observed with breeders fed SM, and consequently, they had the best hatching rate but without any significant difference in the chicks' quality and their weight of internal organs at the hatch. In conclusion, this study demonstrated that using selenium is beneficial, especially in the organic form, which appeared to be more efficient, compared to the inorganic form.

Keywords: Broiler Chicken, Hatching events, Hematology, Selenomethionine, Sodium selenite

INTRODUCTION

Selenium is an essential element in poultry nutrition, and its bioavailability and efficacy depend on its chemical form (Surai et al., 2018). In fact, egg fertility, hatchability, and post-hatch performance are related to the breeds, age of the flock, or incubation conditions of eggs (King'ori, 2011). Moreover, embryonic growth and normal development might also depend on the supply of all required nutrients within the egg, and this supply of nutrients originates from the maternal diet (Wilson, 1997). Nevertheless, nutrient deficiencies or their surplus in poultry nutrition causes serious problems, such as a reduction in chickens' growth and egg production. According to Wilson (1997), the deficiencies or excess of selenium may cause cessation of egg production, and consequently affect embryo development negatively. Due to the relationship between offspring's performance and the maternal diets of poultry, several studies have focused on the improvement of the breeder's nutrition (Kidd, 2003; Aigueperse et al., 2013). As Alagawany et al. (2021a, b) mentioned, delivering a proper amount of minerals to chickens' bodies is important for optimum health and physiological functions. Therefore, there has been a growing interest in the supplementation of poultry diets with some micronutrients. Among the vital trace elements

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required for the body's normal functioning, selenium has a significant role in maintaining optimal health (Muhammad et al., 2016). Hegazy and Adachi (2000) noted that selenium supplementation in poultry feed increases antibody production. Many studies have investigated the comparative effects of the two forms of selenium supplementation in poultry feed on reproductive and growth performances (Mikulski et al., 2009; Delezie et al., 2014; Maja et al., 2018). Traditionally, selenium was used in its inorganic form (sodium selenite) in poultry feeding, but nowadays, there is a growing interest in its organic form. Studies have revealed that the supplementation of organic selenium, especially L-selenomethionine, positively affected poultry performance (Mahmoud and Edens, 2003). Additionally, L-selenomethionine has indicated a higher selenium transfer into the eggs compared to sodium selenite and selenium yeast (Delezie et al., 2014). Similar to this finding, Maja et al. (2018) found that L-selenomethionine supplementation was the best method for the enrichment of selenium in the egg since it could be considered an excellent source of highly bioavailable selenium in the human diet. Despite several studies conducted on the effect of sources of selenium on commercial broilers, there is a paucity of information on the effects of selenium-based diets on broiler breeders, particularly on their hatching and post-hatch quality. Therefore, the present study aimed to compare the performance of breeders fed different sources of selenium in terms of their zootechnical and hematological parameters as well as their progeny performance.

MATERIALS AND METHODS

Study design

The experiment was conducted at Ayodele poultry farm. The experiment lasted 8 weeks, from September to November 2020, with an average temperature of 27°C. A total of 120 breeder hens and 12 roosters of 47 weeks of age from the Sasso strain were assigned to 3 treatment groups. Each treatment had 4 replicates of 10 breeders and 1 rooster each. Four weeks adaptation period was observed during which the experimental broiler breeders were fed a diet without selenium supplementation. The chicks were fed a basal diet (granulated form) without selenium supplementation during an adaptation period of 4 weeks. After that period, two sources of selenium were supplemented to the breeders' diet at 0.2 mg/kg of feed. The two types of selenium were provided by ORFFA additives Bv (Netherlands). The dietary treatments were breeders fed a basal diet with no selenium supplemented (Control), breeders fed a basal diet with sodium selenite (SS), and breeders fed a basal diet with selenomethionine (SM). The roosters were fed a diet with no selenium supplementation. All the breeders were fed according to the recommendation of the Sasso breeder's feeding program (Table 1). The chickens were raised in an opened poultry house, and water was given *ad libitum* to all the treatments.

Table 1. Breeders feed composition

Ingredients (kg)	Breeders	Rooster
Maize	62	56
Wheat bran	8	28.9
Soya bean	3	0
Soya bean meal	9	0
Lysine	0.2	0.4
Methionine	0.1	0.2
Oyster shell	6	2.5
Brewery by product	5	12
Concentrate	6.7	0
TOTAL	100	100
Nutritional content		
Metabolizable energy (Kcal/Kg)	2734.16	2658.33
Crude protein (%)	16.8	12.79
Fat (%)	3.96	4.04
Crude fiber (%)	4.29	5.38
Lysine (%)	0.92	0.7
Methionine (%)	0.45	0.41
Methionine + Cyst (AAS)	0.66	0.58
Calcium (%)	2.1	0.92
Phosphorous (%)	0.51	0.49

Data collection

Performance parameters

From the first week of the dietary selenium supplementation to the end of the trial, daily feed intake and the average weight of eggs laid daily were determined per treatment. The data were then used to calculate the feed conversion ratio (FCR) using the following formulas.

Daily feed intake per bird = Weight of feed giving - weight of the leftover / Number of birds

Average egg weight = Total weight of daily egg collected / Number of eggs collected

FCR = Daily feed intake / Average egg weight

Hematology

At the end of the experiment, 2 ml of blood was collected randomly from the jugular vein of six breeders per treatment using a syringe of 5 cc. The blood samples were collected into a set of sterilized bottles containing

ethylene diamine tetraacetic acid (EDTA) for the determination of hematological parameters, such as packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), and hemoglobin concentration (HGB).

Hatching events evaluation

On the 18 days of incubation, all the eggs were individually candled, and the fertile eggs were transferred to the hatcher under controlled temperature and humidity conditions. From the 457 hours to the 502 hours of incubation, all the eggs were individually checked every 2 hours for internal piping (IP), external piping (EP), or hatching. Then, the time interval between IP-EP, EP-Ha, and IP-Ha was calculated to determine the duration of each event.

Relative organs weight

A total of six hatched chicks per treatment were at hatch. They were weighed individually and were sacrificed through cervical dislocation. Organs, such as liver, yolk sac, and heart, were removed and weighed. Their relative weight was determined through the formula below.

Organ proportion (calculated as relative organ weight) = Weight of organ / Live weight of chick x 100

Statistical analysis

The statistical analysis of the data was performed using Graph pad prism version 8.0.2 as a statistical package. Data obtained were expressed as mean ± SEM and they were compared using a one-way analysis of variance. Tukey test was then used to find means that are significantly different from each other. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Performance and egg weight of chickens

Table 2 shows the effect of selenium-based feed supplementation on the daily feed intake per breeder, egg weight, and FCR of broiler breeders. The dietary treatments significantly affected all mentioned parameters ($p < 0.05$). The feed intake was significantly higher in the breeders fed SS ($p < 0.05$), and the lowest was observed with the control. The egg weight of SM breeders was significantly higher than those of the SS group ($p < 0.05$), but comparable to those of the control group. The FCR of SS breeders was higher than those of SM and the control group, whose values were similar ($p > 0.05$).

Hematological parameters of chicks

The result of the hematology profile is presented in Table 3. The analysis of variance showed that the different sources did not significantly affect most of the leucocyte counts ($p > 0.05$). However, the basophile percentage was significantly influenced by the dietary selenium supplementation ($p < 0.05$). The control group had the highest basophil concentration, while the SS group observed the lowest. The WBC measurements showed no significant difference ($p > 0.05$) between the control and SM group. Nevertheless, the blood cell counts, such as WBC, RBC, HGB, and PLT, were affected by the dietary supplementation of selenium. The WBC, RBC, and HGB were significantly higher ($p < 0.05$) in SM group, while PLT was significantly lower ($p > 0.05$) in the same group.

Duration of hatching events

The incubation parameters duration is shown in Table 4. Selenium did not significantly affect the duration of IP occurrence and the total incubation duration ($p > 0.05$). However, the EP and hatching durations were significantly influenced by the sources of selenium ($p < 0.05$). The EP duration was shorter ($p < 0.05$) in the SM breeders, compared to the control group, while the EP of SS breeders was similar ($p > 0.05$) to that of the control and SM groups. The shortest hatching duration was recorded in the SM group, and the highest value in the SS group ($p < 0.05$). There was no significant difference between SS and control groups in terms of hatching duration ($p > 0.05$).

Chicks' quality at the hatch

The supplementation of breeders' diets with different sources of selenium significantly affected the hatching rate ($p < 0.05$). Chicks from breeders fed the organic selenium had the highest rate, and the control group had the lowest. No difference was observed in the chicks' quality at hatch (Table 5).

Organs' weight of chick at the hatch

The effect of selenium-based feed supplementation on chicks' organ relative weight at hatch is shown in Table 6. The treatments showed no significant difference in chicks' weight at hatch ($p > 0.05$). The bursa, heart, yolk sac, and chicks' weight without yolk sac were not affected by the dietary treatment. However, the relative weight of the liver was significantly influenced by the dietary selenium inclusion in the breeders' diet, with the SM group having the highest relative weight ($p < 0.05$).

Table 2. Effect of diets supplemented with two dietary sources of selenium on daily feed intake per breeders per day, egg weight and feed conversion ratio

Parameters \ Treatments	Control	SS	SM	P-value
FI (g)	118.9 ± 0.64[c]	123.1 ± 0.41[a]	120.6 ± 0.32[b]	0.4084
Egg weight (g)	55.00 ± 0.86[ab]	52.79 ± 0.97[b]	56.92 ± 0.98[a]	0.0107
FCR	2.16 ± 0.01[b]	2.31 ± 0.04[a]	2.10 ± 0.01[b]	<0.0001

[abc] Means in the same row with different subscripts are significantly different at p < 0.05. SS: Sodium Selenite, SM: Selenomethionine: FI: Feed Intake: FCR: Feed conversion ratio

Table 3. Effect of diets supplemented with two dietary sources of selenium on the hematological parameters of broiler chickens

Parameters \ Treatments	Control	SS	SM	P-value
WBC ($10^{/9}$L)	134.5 ± 0.16[b]	151.9 ± 11.70[b]	183.6 ± 1.68[a]	0.0009
Lymp (%)	33.50 ± 5.92	34.75 ± 7.46	44.50 ± 5.62	0.4443
Mono (%)	14.00 ± 3.49	7.50 ± 4.66	4.00 ± 2.45	0.1999
Oesino (%)	9.75 ± 2.25	13.75 ± 4.96	11.50 ± 2.22	0.7144
Neut (%)	28.75 ± 6.97	33.75 ± 8.09	37.75 ± 4.70	0.6521
Baso (%)	3.5 ± 2.26[a]	0.57 ± 0.12[b]	2.00 ± 0.77[ab]	0.0438
HGB (g/dl)	10.90 ± 0.25[b]	11.43 ± 0.67[b]	13.40 ± 0.06[a]	0.0026
RBC (10^{12}/L)	1.93 ± 0.01[c]	2.26 ± 0.15[b]	2.76 ± 0.02[a]	<0.0001
MCV (fL)	133.5 ± 2.27	127.0 ± 1.96	129.9 ± 2.90	0.2203
MCHC (g/dl)	33.53 ± 0.33	33.60 ± 0.22	44.80 ± 12.53	0.8030
PCV (%)	35.25 ± 1.69	31.15 v 1.48	32.48 ± 1.29	0.1978
PLT (%)	178 ± 1.67[a]	117.7 ± 30.02[ab]	63. ± 0.95[b]	0.0020

[abc] Means in the same row with different subscripts are significantly different at p < 0.05. SS: Sodium Selenite, SM: Selenomethionine. WBC: White blood cell, Lymp: Lymphocyte; Mono: Monocyte, Oesino: Oesinophile, Neut: Neutophile, Baso: Basophile, HGB: Hemoglobin, RBC: Red blood cell, MCVMean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, PCV: Packed cell volume, PLT: Platelet

Table 4. Effect of diets supplemented with two dietary sources of selenium on hatching events duration

Parameters (Hours) \ Treatments	Control	SS	SM	P-value
Internal piping	9.95 ± 0.70	10.70 ± 0.83	8.75 ± 0.75	0.2073
External piping	17.19 ± 0.79[a]	15.25 ± 0.79[ab]	13.95 ± 0.92[b]	0.0220
Hatching	23.38 ± 0.83[a]	23.74 ± 0.79[a]	19.62 ± 1.32[b]	0.0078
Incubation	486.9 ± 0.81	486.0 ± 0.75	485.2 ± 0.78	0.3357

[ab] Means in the same row with different subscripts are significantly different at p < 0.05. SS: Sodium Selenite, SM: Selenomethionine, FI: Feed Intake, FCR: Feed conversion ratio.

Table 5. Effect of diets supplemented with two dietary sources of selenium on chicks' quality at hatch

Parameters (Hours) \ Treatments	Control	SS	SM	P-value
Hatching rate (%)	56.10 ± 0.06[c]	83.12 ± 0.01[b]	88.00 ± 0.23[a]	<0.0001
Chicks' quality (%)	90.20 ± 2.26	88.80 ± 1.72	87.00 ± 2.65	0.6451

[abc] Means in the same row with different subscripts are significantly different at p < 0.05. SS: Sodium Selenite, SM: Selenomethionine, FI: Feed Intake, FCR: Feed conversion ratio

Table 6. Effect of diets supplemented with two dietary sources of selenium on chicks' organs relative weight at hatch

Parameters (Hours) \ Treatments	Control	SS	SM	P-value
Weight at hatch (g)	38.66 ± 3.20	35.95 ± 1.87	37.17 ± 4.62	0.8566
Bursa (g)	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.8809
Heart (g)	0.79 ± 0.05	0.63 ± 0.01	0.77 ± 0.13	0.3708
Liver (g)	2.36 ± 0.09[a]	1.85 ± 0.13[b]	2.20 ± 0.15[a]	0.049
Yolk (g)	10.96 ± 1.08	13.71 ± 1.34	12.58 ± 1.71	0.4299
Chick weight without yolk sac (g)	81.16 ± 1.52	80.21 ± 0.79	80.35 ± 2.56	0.9212

[ab] Means in the same row with different subscripts are significantly different at p < 0.05. SS: Sodium Selenite, SM: Selenomethionine

DISCUSSION

The present study aimed to compare the effect of broiler breeders' diets supplemented with two sources of selenium (organic and inorganic) on the zootechnical and hematology parameters, hatching parameters, and chicks' internal organs' relative weight at the hatch. Generally, dietary selenium supplementation could significantly affect the performance parameters. Although breeders fed SS had the highest feed intake, their egg weight was the lowest. The best FCR was observed with breeders fed with the organic selenium indicating that the metabolism pathways of the two kinds of selenium are different, as previously shown by several other authors (Lyons et al., 2007; Surai and Fisinin, 2014; Brandt-Kjelsen et al., 2017). As stated by Marković et al. (2018), the organic forms of selenium-containing selenomethionine (Se-Met) and selenocysteine (Se-Cys) play a key role in biological processes since they are more active and less toxic, have higher bioavailability, and accumulate at higher levels in all tissues, compared to the inorganic form of selenium. Similarly, Whanger (2002) mentions that selenium is better absorbed in the organic form. Selenomethionine is made of methionine (an essential amino acid precursor of protein synthesis) and selenium, with the mean concentration of proteins being 12.5 g per 100 g of whole raw fresh egg (Réhault-Godbert et al., 2019). Although breeders fed with SM consumed a lower amount of feed, the average weight of their eggs was higher. The methionine contained in the SM probably participated in the constitution of the egg protein. However, the obtained results of the current study are not in accordance with that of Attia et al. (2010), indicating no significant difference in the sources of selenium but a significant difference in the level of supplementation while comparing the effect of the breeders' diet supplemented with SM and SS on the egg weight. In the present study, selenium supplementation significantly affected the total RBC, WBC, and HGB, and the highest values were obtained in the Selenium groups, especially the SM group. This result demonstrated that selenium supplementation had no adverse effect on the health status of the breeders. In contrast, it improved their well-being, especially those fed diets supplemented with SM. This was illustrated by the best zootechnical performances observed with the SM group. In addition, it has been shown by Altan et al. (2000) that the exposure of broilers to a stress condition, especially temperature, decreases blood parameter concentration. The highest concentrations observed in the present study with the selenium group revealed the anti-stress role of selenium. However, the findings of the present study contradicted those of Liu et al. (2019), indicating a non-significant effect of selenium supplementation on hematological parameters despite its high level (3 mg of selenium yeast per kg of feed). The difference in the results might be attributed to the selenium content of the basal diet. According to Gupta and Gupta (2000) and Surai (2007), selenium content in different soils usually varies from 0.1 to 2 mg/kg but with slight deviations from this indicator. Thus, tropical and subtropical soils contain relatively high levels of selenium greater than 0.30 mg/kg. The average selenium concentration in temperate and desert soils is between 0.14 and 0.30 mg/kg, while its concentration in a temperate and humid climate is deficient (less than 0.12 mg/kg, Tan et al., 2002).

The improved performance observed with the organic selenium in terms of the performance and hematological parameters significantly influenced the incubation performance. The hatching process is a stressful period for hatching chicks. This stress is accompanied by the production of free radicals, which alter the normal progression of the hatching process. The best hatching process observed with the SM group was indicative of the antioxidant capacity attributed to organic selenium, although no significant effect between organic and inorganic selenium was observed on the hatched chicks' quality.

The comparison of diets supplemented with different sources of selenium showed no significant difference in the internal organs except the liver's relative weight, which was significantly higher in the SM group. Consequently, chicks from this group will probably perform well after hatch. According to Zaefarian et al. (2019), the heavy liver size of broilers during the early post-hatch leads to efficient nutrient metabolization due to lower feed intake and endogenous enzyme secretion.

CONCLUSION

The present study evaluating the efficiency of broiler diets supplemented with two sources of selenium showed that the zootechnical and hatching egg performance of chicks has improved with the organic selenium. In fact, the organic form had a positive effect on the hematological parameters, leading to a positive effect on feed conversion ratio and then incubation parameters, such as hatching events length and hatching rate. Briefly, this study demonstrated that organic selenium supplementation has an impact on the physiological activities of broiler

breeders. This could indicate a probable transfer of selenium in the hatching eggs and its effect on the developing embryo. Therefore, there is a need to evaluate the egg quality, the welfare of the developing embryo, and the subsequent performance of the progeny of broiler breeders fed organic selenium, compared to non-organic selenium supplementation.

DECLARATION

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Authors' contribution

This research work was designed and carried out by Kwassi Tona with the contributions of the above-mentioned co-authors. Wéré Pitala validated the protocol and the final version of the manuscript, Souglman Fiougou helped with fieldwork and data collection, Amen Ayawo Nenonene contributed to the design and statistical analysis, while Emmanuel OKE and Adeboye Fafiou assisted in the revision of the manuscript. All authors read and approved the final manuscript.

Competing interests

The author declares no existence of any form of conflict of interest related to this manuscript. There are also no financial, personal, or other relationships with other people or organizations related to this study.

Ethical consideration

All authors have checked the ethical issue, such as plagiarism, consent to publish, data fabrication and falsification, and redundancy.

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Effects of Xylanase Supplementation on the Performance, Nutrient Digestibility, and Digestive Organ Profiles of Broiler Chickens: A Meta-analysis

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ABSTRACT

Enzymes supplementation in broiler feeding is commonly applied to optimize animal feed utilization and reduce feed production costs. One of the enzymes widely used in the broiler industry is xylanase which breaks down complex fibrous compounds in feed, such as nonstarch polysaccharides, to simpler utilizable sugar molecules. However, the effects of xylanase enzymes on broiler growth performance, nutrient digestibility, and organ function in broiler chickens were variable and inconclusive. Therefore, the current study aimed to evaluate the effect of the xylanase enzyme in feed on the performance, nutrient digestibility, and digestive function of parrots using a meta-analysis approach. A dataset of 140 points obtained from 53 articles was analyzed using a mixed model methodology. The results showed that the xylanase enzyme supplementation increased the broiler's body weight gain and decreased feed consumption and feed conversion ratio. In addition, xylanase supplementation also increased nutrient digestibility, such as dry matter, crude protein, starch, gross energy, fat, phosphorus, and calcium. Concerning broiler organ weights, the xylanase supplementation in broiler feed significantly reduced the weight of the duodenum, small intestine, and relative length of the duodenum, jejunum, and ileum. Xylanase supplementation also tended to reduce the relative weight of the proventriculus. The results also showed a negative response to the crypt depth ileum of broiler due to xylanase supplementation. It can be concluded that xylanase supplementation improves the performance, nutrient digestibility, and digestive function of broiler chickens.

Keywords: Broiler chickens, Nonstarch polysaccharide, Performance, Xylanase enzyme

INTRODUCTION

The utilization and efficiency of feeds are critical factors for improving the performance and production rate in the livestock industry, including the broiler chicken (Masey-O'Neill et al., 2014). High fiber content in feedstuffs is a major issue in broiler chicken feeding that affects feed utilization and efficiency (Liu and Kim, 2017). Hence, it is difficult to optimize the expected result of broiler production due to the inability of broiler chickens to utilize feed compounds and increased production costs resulting from low feed efficiency (Liu and Kim, 2017).

Some dietary interventions have been applied to increase feed nutritional values and improve livestock production efficiently (Hidayat et al., 2021). Researchers' interests are currently focusing on enzyme utilization supplemented in the ration to increase feed nutritional values and reduce livestock production costs (Francesch et al., 2012). For instance, nonstarch polysaccharide (NSP), a complex fiber compound in feedstuff, is difficult to digest properly by monogastric animals, such as poultry (Craig et al., 2019). The insufficient ability of poultry to digest NSP compounds is due to the lack of specific enzymes in their digestive tract that can dissolve undigestible fiber bounds

like NSP, especially in broiler chickens. Hence, some studies reported utilizing the xylanase enzyme in the broilers' ration to increase broiler chickens' feed nutritional values and digestibility (Gonzalez-Ortiz et al., 2017; Lee et al., 2019). Xylanase is an enzyme that can dissociate plant cell walls and reduce hemicellulose integrity and the viscosity of feed consumed in the broiler digestive system. Thus, the released feed nutrients may be optimally utilized and improve broilers' performance (Gonzalez-Ortiz et al., 2017).

Besides reducing the viscosity of intestinal contents, the xylanase enzyme is also known to possess the growth-promoting effect and appears to be partly related to the modulation of gut microflora (Craig et al., 2019). Such improvement may increase the feed efficiency of broiler chickens (Sarangi et al., 2016). However, studies concerning the effects of xylanase on broiler chickens' performance, nutrient digestibility, and digestive organs are varied and remain inconclusive (Olukosi et al., 2020). It is necessary to summarize the published data to determine the effects of xylanase on broiler chickens. Therefore, the current study evaluated the impact of dietary xylanase supplementation on broiler chickens' performance, internal organs, and nutrient digestibility by summarizing research data from various published articles.

MATERIALS AND METHODS

Database development

The database was built from published articles related to the study of the xylanase utilization on broiler chickens. Articles were obtained from Scopus, Google Scholar, NCBI, and Science Direct search engines using "xylanase", "performance", "fiber", and "broiler chicken" keywords. Articles that were included in the database must accede to the following criteria. They described experiments on broiler chickens, treatments were based on the levels of xylanase supplementation in basal feeds, and they must include data about broilers' growth performance (feed intake and daily gain), organ weight and/or internal organ morphometrics, and the articles were written in English. Data from these articles were rigorously selected to be included in the database following some selection stages (Figure 1).

A total of 140 data points representing treatments from 53 articles were included in the database (Table 1). Articles expressed the xylanase supplementation unit as the international unit (IU) values were homogenized and expressed as per 1000 IU (n / 1000 IU). The data included performance parameters (body weight gain, feed consumption, feed conversion ratio [FCR]), nutrient digestibility (dry matter [DM], gross energy, fat, starch, crude protein [CP], phosphorus, calcium digestibility), relative weight of internal organs and digestive tract (proventriculus, liver, pancreas, gizzard, duodenum, jejunum, ileum, small intestine, cecum), relative lengths of the duodenum, jejunum, ileum, cecum, and intestinal morphometrics (villus height [VH], crypt depth [CD], and VH: CD of the duodenum, jejunum, and ileum). Data expressed in different units were converted and adjusted into that of specified units to allow direct analysis within specified parameters. The statistical summary of the database included in the meta-analysis study is presented in Table 2.

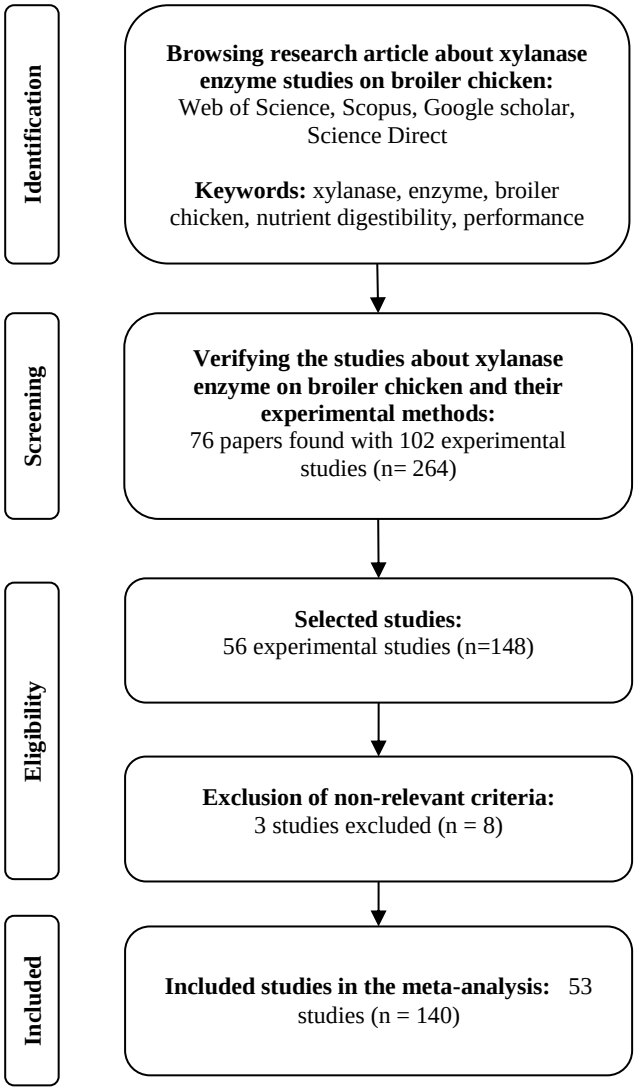

Figure 1. Selection of studies related to the effect of xylanase enzyme on broiler chickens

Table 1. The selected and used studies on the effects of dietary supplementation of xylanase on the performance, nutrient digestibility, and digestive organ profiles of broiler chickens

No.	References	Period (day)	Basal feed	Broiler sex	Xylanase dosage (10^3 IU/kg DM feed)		
1	Selle et al. (2003)	4-24	Wheat	Male	0	-	5.49
2	Wu and Ravindran (2004)	1-35	Wheat	Male	0	-	1.00
3	Wu et al. (2004)	1-21	Wheat	Male	0	-	1.00
4	Wu et al. (2004)	1-21	Wheat	Male	0	-	1.00
5	Olukosi et al. (2007b)	1-21	Wheat	-	0	-	32.0
6	Amerah et al. (2008)	1-21	Wheat	Male	0	-	1.00
7	Woyengo et al. (2008)	1-21	Wheat	Male	0	-	2.50
8	Yang et al. (2008)	8-21	Wheat	-	0	-	1.00
9	Amerah et al. (2009)	1-21	Wheat	Male	0	-	1.00
10	Lu et al. (2009)	1-35	Corn	Male	0	-	2.00
11	Luo et al. (2009)	1-22	Wheat	Male	0	-	5.00
12	Cowieson et al. (2010)	1-22	Corn	Male	0	-	16.0
13	Liu et al. (2011)	22-42	Corn	Mixed	0	-	3.60
14	Nian et al. (2011)	1-30	Wheat	Male	0	-	4.00
15	Walk et al. (2011)	1-18	Corn	Male	0	-	1.20
16	Amerah et al. (2012)	1-22	Wheat	Male	0	-	2.00
17	Esmaelipour et al. (2012)	1-24	Wheat	Male	0	-	1.00
18	Liu et al. (2012)	1-21	Wheat	Male	0	-	5.50
19	Masey-O'Neill et al. (2012)	1-18	Corn	Mixed	0	-	16.0
20	Singh et al. (2012)	1-22	Corn	Male	0	-	16.0
21	Barekatain et al. (2013a)	1-35	Corn	Male	0	-	1.00
22	Barekatain et al. (2013b)	1-21	Corn	Male	0	-	1.00
23	Cowieson and and Masey O'neill (2013)	1-49	Wheat	Male	0	-	16.0
24	Gehring et al. (2013)	1-25	Corn	Female	0	-	32.0
25	Kiarie et al. (2014)	1-22	Wheat	Male	0	-	1.25
26	Masey-O'Neill et al. (2014)	1-49	Corn	Male	0	-	32.0
27	Zhang et al. (2014)	7-21	Wheat	Male	0	-	3.20
28	Pirgozliev et al. (2015)	7-21	Soybean meal	Male	0	-	2.00
29	Gonzalez-Ortiz et al. (2016)	1-24	Wheat	Male	0	-	16.0
30	Kim et al. (2016)	14-35	Corn	Male	0	-	6.00
31	Amerah et al. (2017)	1-22	Corn	Male	0	-	2.00
32	dos Santos et al. (2017)	1-24	Sorghum	Mixed	0	-	16.0
33	Gonzalez-Ortiz et al. (2017)	1-22	Wheat	Male	0	-	16.0
34	Kiarie et al. (2017)	1-21	Wheat	Male	0	-	5.00
35	Liu and Kim (2017)	1-35	Wheat	Male	0	-	5.63
36	Mabelebele et al. (2017)	1-21	Sorghum	Female	0	-	1.60
37	Pakel et al. (2017)	7-21	Corn	Male	0	-	0.80
38	Tang et al. (2017)	1-22	Barley	Mixed	0	-	16.0
39	Ghayour-Najafabadi et al. (2018)	1-22	Wheat	Male	0	-	1.00
40	Lee et al. (2018)	1-22	Wheat	Male	0	-	16.0
41	Moss et al. (2018)	10-21	Canola	Male	0	-	2.00
42	Widodo et al. (2018)	1-21	Soybean meal	Male	0	-	16.0
43	Arczewska-Wlosek et al. (2019)	1-22	Corn	Male	0	-	1.00
44	Craig et al. (2019)	1-22	Wheat	Male	0	-	32.0
45	Gonzalez-Ortiz et al. (2019)	1-24	Wheat	Male	0	-	16.0
46	Lee et al. (2019)	1-32	Corn	Mixed	0	-	11.3
47	Olukosi and Bedford (2019)	1-28	Wheat	Male	0	-	16.0
48	Craig et al. (2020a)	1-21	Wheat	Male	0	-	16.0
49	Craig et al. (2020b)	1-28	Wheat	Male	0	-	32.0
50	Melo-Durán et al. (2020)	1-21	Corn	Male	0	-	16.0
51	Olukosi et al. (2020)	1-28	Wheat	Male	0	-	16.0
52	Pirgozlive et al. (2021)	7-21	Wheat	Female	0	-	0.10
53	Rabello et al. (2021)	1-49	Corn	Male	0	-	32.0

Table 2. Descriptive statistics of the observed parameters in broiler chickens by using xylanase supplementation in diet

Response Parameters	N	Mean	SEM	Min	Max
Performance and feed intake					
BWG (g/chick)	138	1558	88.5	285	4206
ADG (g/d/chick)	138	50.2	1.71	21.0	124
FI (g/chick)	138	2574	156	551	7502
ADFI (g/d/chick)	138	82.0	3.35	27.0	207
FCR (g/g)	138	1.60	0.02	1.03	2.29
Nutrient digestibility					
DM (%)	46	71.3	0.95	60.4	85.0
CP (%)	35	70.2	1.67	55.5	83.0
Starch (%)	19	77.8	6.64	0.92	98.1
Nitrogen (%)	24	79.0	1.50	64.0	90.0
Fat (%)	15	81.6	2.20	66.0	91.3
GE (%)	33	70.8	1.14	61.3	82.1
Phosphorus (%)	20	46.7	2.13	32.4	66.2
Calcium (%)	18	49.7	3.32	17.8	65.0
Relative weight					
Proventriculus (%)	12	0.40	0.03	0.28	0.59
Liver (%)	20	2.65	0.18	0.93	3.80
Pancreas (%)	22	0.29	0.02	0.14	0.46
Gizzard (%)	31	1.50	0.08	0.87	2.33
Duodenum (%)	30	0.67	0.05	0.42	1.17
Jejunum (%)	30	1.17	0.06	0.75	2.08
Ileum (%)	30	0.95	0.05	0.62	1.74
Small intestine (%)	36	3.40	0.26	1.93	6.90
Cecum (%)	26	0.34	0.03	0.12	0.70
Relative length					
Duodenum (cm/100g BW)	16	2.27	0.14	1.30	3.16
Jejunum (cm/100g BW)	16	6.07	0.37	3.63	7.87
Ileum (cm/100g BW)	16	6.08	0.37	3.71	7.85
Small intestine (cm/100g BW)	16	14	0.87	8.69	18.8
Cecum (cm/100g BW)	22	1.39	0.06	0.88	1.72
Villus Height (VH)					
Duodenum (µm)	22	1257	90.9	772	1949
Jejunum (µm)	30	809	41.5	354	1467
Ileum (µm)	22	626	48.4	355	1142
Crypt Depth (CD)					
Duodenum (µm)	22	249	33.6	56.8	480
Jejunum (µm)	30	204	23.0	38.6	402
Ileum (µm)	22	150	16.4	37.2	257
VH: CD					
Duodenum (µm)	16	5.61	1.51	0.08	18.0
Jejunum (µm)	24	5.68	1.34	0.12	25.5
Ileum (µm)	20	5.58	1.10	0.13	14.5

N: Number

Statistical analysis

The recorded database was processed statistically using the mixed model method with SAS software version 9.1 (St-Pierre, 2001; Sauvant et al., 2008). Different studies were assigned as random effects, and the dose of xylanase enzyme was assigned as a fixed effect following Hidayat et al. (2021) and Yanza et al. (2021b) modification methods, in which the effects of xylanase were assessed to have a relationship with factors, such as type of basal diets (barley, canola meal, corn, sorghum, soybean meal, and wheat) and sex (male and female). The mathematical model used in the present meta-analysis was accomplished as follows:

$$Y_{ij} = \mu + s_i + \tau_j + s\tau_{ij} + B_0 + B_1X_{ij} + B_2X_{2ij} + b_iX_{ij} + e_{ij}$$

Where, Y_{ij} is the dependent variable, µ denotes the average of all data,, s_i signifies the random effect of the i-th trial, τ_j is defined as the fixed effect of the jth-level factor, sτij is the random interaction between the i-th test and the jth-level factor, B_0 indicates the overall intercept in all experiments (fixed effect), B_1 refers to the linear regression coefficient Y on X (fixed effect), X_{ij} suggests the value of the continuous predictor variable

(xylanase/1000 IU), b_i is the random effect of the study on the regression coefficient Y on X in the study -i, and e_{ij} show an unexplained residual error.

Due to the qualitative information, the class determination was based on the sex factor and the type of basal diet. Different studies were considered as random effects in the model. The number of replicates in the studies was determined based on the statement of weight available in the SAS as performed by Yanza et al. (2021a). The variables were considered significant at $p < 0.05$ and considered to have a tendency at $0.05 < p < 0.10$.

RESULTS

Supplementation of xylanase enzyme in ration positively affected broiler chickens' growth performance and nutrient digestibility (Tables 3 and 4). The total body weight gain (BWG) and average daily gain (ADG) of broiler chickens increased by a quadratic response ($p < 0.05$). However, xylanase supplementation reduced the broiler chickens' feed consumption when expressed as a total feed intake (FI; $p < 0.05$) and tended to reduce when linearly expressed as an average daily feed intake (ADFI; $p < 0.10$). Consequently, xylanase supplementation in broiler's ration reduced FCR. The effects of xylanase supplementation on FCR interacted with the type of diet ($p < 0.05$).

Xylanase supplementation enhanced nutrient digestibility, such as DM, CP, and starch by a quadratic response ($p < 0.05$). The xylanase supplementation, however, did not alter fat digestibility. Digestibility of phosphorus, calcium, and total gross energy digestibility (GED) were also elevated by xylanase supplementation by a linear response ($p < 0.05$). The effects of xylanase on phosphorus and calcium digestibility interacted with the type of diet ($p < 0.05$).

The present study showed that xylanase supplementation tended to negatively affect proventriculus ($p < 0.10$) in a quadratic manner, which had an interaction with the type of diet ($p < 0.05$; Table 5). Furthermore, the xylanase supplementation negatively affected the weight of the duodenum ($p < 0.05$) and small intestine ($p < 0.05$), in a quadratic and linear manner, respectively. The xylanase supplementation negatively influenced duodenum, jejunum, and ileum lengths in a quadratic manner ($p < 0.05$), while the small intestine length was reduced linearly ($p < 0.05$). The xylanase supplementation did not affect VH, VH: CD ratio of duodenum, jejunum, and ileum, and the CD of duodenum and jejunum (Table 6). However, xylanase supplementation lowered the CD of the ileum in a linear manner ($p < 0.05$).

DISCUSSION

Effects of xylanase on performance and nutrient digestibility of broiler chickens

The inclusion level of the feed ingredients with relatively high polysaccharide content, such as xylan and pentosan, may increase due to the availability of exogenous enzymes used in animal feed (Olukosi et al., 2007a). However, the effects of exogenous enzymes on livestock animals, such as broiler chickens, depend on utilizable nutrients contained in the dietary ration, species, age of the animal, level of antinutrients in feed ingredients, or a combination of these and other factors (Olukosi et al., 2007b). Nonstarch polysaccharide in broiler chicken feed is known to be hydrolyzed using the xylanase enzyme. In addition, it randomly cleaves the arabinoxylan backbone, generating unsubstituted or branched xylooligosaccharides, consequently increasing the polysaccharide substrate's bioavailability (Arczewska-Wlosek et al., 2019; Lee et al., 2017). Recently, results concerning the effects of xylanase on broiler chickens have been varied. Thus, it needs a systematic approach to obtain robust conclusions on several parameters. Hence, the present study was expected to determine the effects of the xylanase supplementation on the critical parameters in broiler chicken nutrition, such as performance (weight gain, feed consumption, and FCR), internal organs, and nutrient digestibility evaluated from published articles.

Xylanase is an exogenous degrading enzyme that can increase broiler chickens' bioavailability of dietary nutrients. The findings of the present study indicated an increased body weight of broiler chickens and decreased FCR, followed by increased levels of xylanase supplementation in feed ingredients. Therefore, it can be assumed that broiler chickens fed with ration supplemented xylanase may improve their performance (Liu and Kim, 2017). Previous studies reported positive effects of xylanase on broiler performance, such as body weight gain, feed intake, and FCR, which further positively increase the nutrient digestibility of broiler chickens (Cowieson, 2010; Kiarie et al., 2014). Xylanase degrades the polysaccharides structure of plant cell walls, especially NSP that is xylan, into xylose and releases other simple sugars or nutrients. Hence, the broiler chickens can readily utilize the released sugars or nutrients for further metabolism (Dornez e al., 2009).

Table 3. The effect of xylanase supplementation (per 1000 IU) on performance and feed intake in broiler chickens

Parameters	N	Model	Parameter estimate					Model statistics		Interaction		Trends
			Intercept	SE Intercept	Slope	SE Slope	P-value	RMSE	AIC	Enzyme × Diet	Enzyme × Sex	↑ / ↓
BWG (g/chick)	138	Q	1518	143	4.92	1.43	<0.001	33.02	1798	NS	NS	↑
					-0.12	0.05	0.022					
ADG (g/chick)	138	Q	49.6	2.77	0.15	0.046	0.001	1.05	820	NS	NS	↑
					-0.003	0.002	0.042					
FI (g/chick)	138	L	2525	253	-1.93	0.82	0.021	48.51	1917	NS	NS	↓
ADFI (g/d/chick)	138	L	81.3	5.47	-0.049	0.028	0.078	1.64	951	NS	NS	↓
FCR (g/g)	138	L	1.61	0.04	-0.003	0.0006	<0.001	0.04	-204	0.044	0.080	↓

BWG: Body weight gain, ADG: Average daily gain, FI: Feed intake, ADFI: Average daily feed intake, FCR: Feed conversion ratio, L: Linear, Q: Quadratic, SE, Standard error, RMSE: Root mean square of errors, AIC: Akaike information criterion, L: Linear response, ↑ / ↓: symbol to indicate increasing/decreasing effect of treatment, The model tends to be significant at $p < 0.1$, significant at $p < 0.05$, NS: Not significant, N: Number

Table 4. The effect of xylanase supplementation (per 1000 IU) on nutrient digestibility in broiler chickens

Parameters	N	Model	Parameter estimate					Model statistics		Interaction		Trends
			Intercept	SE Intercept	Slope	SE Slope	P-value	RMSE	AIC	Enzyme × Diet	Enzyme × Sex	↑ / ↓
DM (%)	46	Q	70.2	1.64	0.34	0.14	0.020	1.71	261	NS	NS	↑
					-0.011	0.004	0.025					
CP (%)	35	Q	69.4	3.11	0.67	0.17	<0.001	0.85	173.4	NS	NS	↑
					-0.026	0.012	0.049					
Starch (%)	19	Q	74.7	11.0	1.16	0.33	0.006	0.80	123	0.008	NS	↑
					-0.067	0.021	0.011					
Fat (%)	15	L	80.7	3.37	0.41	0.69	NS	2.36	94.4	0.019	0.001	↑
GE (%)	33	Q	70.1	1.83	0.44	0.10	<0.001	1.09	165	NS	NS	↑
Phosphorus (%)	20	L	45.2	3.64	0.44	0.12	0.003	1.87	119.7	0.025	NS	↑
Calcium (%)	18	L	46.6	6.29	0.55	0.16	0.005	2.51	117.3	NS	NS	↑

DM: Dry matter, CP: Crude protein, GE: Gross energy, L: Linear, Q: Quadratic, SE: Standard error, RMSE: Root mean square of errors, AIC: Akaike information criterion, ↑ / ↓: symbol to indicate increasing/decreasing effect of treatment. The model tends to be significant at $p < 0.1$, significant at $p < 0.05$, very significant at $p < 0.001$, NS: Not significant, N: Number

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Table 5. The effect of xylanase supplementation (per 1000 IU) on relative weight and length of digestive organs in broiler chickens

Parameters	N	Model	Parameter estimate					Model statistics		Interaction		Trends
			Intercept	SE Intercept	Slope	SE Slope	P-value	RMSE	AIC	Enzyme × Diet	Enzyme × Sex	↑ / ↓
Relative Weight (%)												
Proventriculus	12	Q	0.4	0.05	-0.03	0.01	0.088	0.01	-9.7	0.003	NS	↓
					0.002	0.001	0.085					
Liver	20	L	2.59	0.33	0.006	0.007	NS	0.12	23.5	NS	NS	↑
Pancreas	22	L	0.3	0.03	0.00004	0.002	NS	0.02	-55.4	NS	NS	↑
Gizzard	31	L	1.53	0.13	-0.002	0.004	NS	0.11	15.5	NS	NS	↓
Duodenum	30	Q	0.74	0.08	-0.028	0.013	0.048	0.02	-40.1	NS	NS	↓
					0.0018	0.0008	0.044					
Jejunum	30	L	1.24	0.1	0.0006	0.003	NS	0.05	-17	NS	NS	↑
Ileum	30	L	0.99	0.09	0.0022	0.003	NS	0.05	-18.5	NS	NS	↑
Small intestine	30	L	3.48	0.51	-0.021	0.007	0.009	0.11	36.1	NS	NS	↓
Cecum	26	L	0.36	0.06	-0.0002	0.002	NS	0.02	-58.6	NS	0.09	↓
Relative Length (cm/100 g BW)												
Duodenum	16	Q	2.31	0.24	-0.2	0.08	0.042	0.1	25.5	NS	NS	↓
					0.013	0.005	0.04					
Jejunum	16	Q	6.04	0.61	-0.39	0.14	0.024	0.17	43.4	NS	NS	↓
					0.025	0.009	0.024					
Ileum	16	Q	6.07	0.62	-0.42	0.13	0.015	0.17	42.7	NS	NS	↓
					0.026	0.009	0.015					
Small intestine	14	L	15.5	1.4	-0.84	0.35	0.044	0.5	49.4	NS	NS	↓
Cecum	22	L	1.35	0.1	0.001	0.006	NS	0.05	-13.6	NS	NS	↑

BW: Body weight, L: Linear, Q: Quadratic, SE: Standard error, RMSE: Root mean square of errors, AIC: Akaike information criterion, NS: Not significant, N: Number, ↑ / ↓: symbol to indicate increasing/decreasing effect of treatment. The model tends to be significant at $p < 0.10$, significant at $p < 0.05$, very significant at $p < 0.001$, NS: Not significant.

Table 6. The effect of xylanase supplementation (per 1000 IU) on morphometric traits of broiler's digestive tract

Parameters	N	Model	Parameter estimate					Model statistics		Interaction		Trends
			Intercept	SE Intercept	Slope	SE Slope	P-value	RMSE	AIC	Enzyme × Diet	Enzyme × Sex	↑ / ↓
Villus height (VH)												
Duodenum (µm)	22	L	1357	159	3.27	3.58	NS	31.39	252	NS	NS	↑
Jejunum (µm)	30	L	805	79.4	6.06	6.29	NS	57.2	362	NS	NS	↑
Ileum (µm)	22	L	678	85	2.62	3.1	NS	37.78	254	NS	NS	↑
Crypt depth (CD)												
Duodenum (µm)	22	L	233	58.8	-0.25	0.69	NS	6.06	192	NS	NS	↓
Jejunum (µm)	30	L	189	40.8	-1.46	1.23	NS	11.09	283	NS	NS	↓
Ileum (µm)	22	L	144	28.5	-1.08	0.42	0.024	5.12	185.4	0.041	NS	↓
VH: CD												
Duodenum (µm)	16	L	5.08	3	0.03	0.11	NS	0.96	74.1	NS	NS	↑
Jejunum (µm)	24	L	5.69	2.66	-0.02	0.19	NS	1.7	127.4	NS	NS	↓
Ileum (µm)	20	L	5.68	1.99	0.05	0.09	NS	1.08	97.3	NS	NS	↑

L: Linear, Q: Quadratic, SE: Standard error, RMSE: Root mean square of errors, AIC: Akaike information criterion, N: Number; ↑ / ↓: symbol to indicate increasing/decreasing effect of treatment. The model tends to be significant at $p < 0.1$, significant at $p < 0.05$, very significant at $p < 0.001$, NS: Not significant;

However, the efficacy of xylanase to break down NSP structures are different, depending on the type of feed sources and NSP contained in feed materials. For example, poultry prefers to consume cereals that typically have high NSP. However, the polysaccharide composition and the solidness of NSP structural bounds of cereals are different and might influence animal nutrient digestibility (Gonzalez-Ortiz et al., 2017; Bryszak et al., 2020). For example, the corn diet contained 8.7% and 65.2% of NSP and starch, respectively, which is more rapidly digested by the broiler chicken, compared to wheat, which included 10.9% and 65.2% of NSP and starch, respectively (Peron and Amerah, 2012). Therefore, by adding xylanase to both types of feed, broiler chickens' digestibility might increase. However, xylanase seems more effective in dissociating NSP and fiber molecular structures in corn than in wheat. It was studied that broilers fed corn had a higher fiber and phosphorus digestibility than broilers fed with wheat in basal diets (Cowieson et al., 2010; Amerah et al., 2017; Kiarie et al., 2017; Liu and Kim, 2017). According to Bedford (2000), xylanase supplementation can reduce hemicellulose integrity and release previously encapsulated nutrients to improve digestive function and animal performance. However, broiler chicken's feed intake and FCR in the present meta-analysis were reduced by xylanase supplementation. Such xylanase mode of action may increase released nutrients and reduce hemicellulose integrity, and viscosity of feed consumed in the broiler chicken intestines (Kiarie et al., 2014; Khadem et al., 2016). Hence, available nutrients can be optimally absorbed in the broiler chicken hindgut. Nutrient utilization can improve broiler chickens' performance indicated by the increased weight gain and reduced feed intake or simplified by the FCR value (Kiarie et al., 2014).

Results also showed that xylanase supplementation interacted with FCR and fat digestibility in broiler chickens of different sexes. It was reported that the body weight gain, feed intake, and FCR of male chickens were higher than that of female chickens, indicating higher susceptibility of female chickens to disease than males (Ozkan et al., 2010; Quinteiro-Filho et al., 2010; Qurniawan et al., 2016).

The present study indicated that xylanase increased broiler chickens' nutrient digestibility, such as DM, CP, starch, fat, gross energy, phosphorus, and calcium. Kim et al. (2016) explained that adding xylanase to feed ration would increase the accessibility of encapsulated nutrients in the cell wall by demolishing plant cell wall structures through the arabinoxylan degradation. Therefore, it is assumed that xylanase is activated when consumed feed is delivered to the small intestine. Thus, undegraded nutrients in the previous digestive track, with the activated xylanase enzyme, degraded nutrients were readily absorbed in the hindgut. According to Francesch et al. (2012), the xylanase enzyme in poultry feed generally reduces digesta viscosity and increases the digestibility of nutrients. As reported, NSP in wheat may exacerbate endogenous amino acid secretion, suppressing amino acid digestibility (Angkanaporn et al., 1994; Liu and Kim, 2017). Accordingly, xylanase can reduce intestinal viscosity and endogenous amino acid secretion, release trapped nutrients, and increase cell wall permeability to absorb utilizable nutrients (Liu and Kim, 2017).

Effects of xylanase on weights and morphometrics of broiler chicken's digestive organs

In this study, Xylanase supplementation in broiler chicken feed showed no effect on the observed organ weights, such as pancreas, gizzard, jejunum, ileum, and cecum. However, the relative weight of the proventriculus, duodenum, and small intestine as well as the relative length of the duodenum, jejunum, ileum, and small intestine decreased with the xylanase supplementation. However, the present study results showed that high fibrous in feed ration can be digested properly due to exogenous enzymes, such as xylanase. Previous studies revealed that fiber or NSP components in poultry feed ration could influence intestinal development, especially the gizzard, and consequently digestibility (Hetland et al., 2004). Yasar and Forbes (2000) reported that the tissue lining the gizzard and the thickness of the gizzard glands of broiler chickens could be reduced by adding enzymes to the feed. On the contrary, Gonzalez-Ortiz et al. (2017) confirmed that no change in organ size was observed in any organs as measured by xylanase enzyme supplementation. Although gizzard weight slightly decreased due to the xylanase supplementation, it was still in the acceptable weight range.

The excessive fiber in feed ingredients may impact digestive organ size with the increased thickness of the apparent organ layer; thus, the weight of some digestive organs increases. In contrast, the current results found that xylanase may not interfere with liver activity in the detoxification process and negatively affect liver size (Septinar et al., 2021). This study revealed that xylanase positively affects metabolic processes so that the degraded nutrients can be well absorbed in the small intestine. Therefore, the cecum of broilers does not need to be burdened because of the workload of the digestive process,

which can affect the size of the cecum. With xylanase supplementation, the indigestible fiber becomes more easily digested. Therefore, increasing digestive metabolism has no detrimental effect on organ function (Sharifi et al., 2012).

Additionally, most organs are believed to work properly even though the xylanase has a detrimental effect on weight and relative length of specified digestive organs as indicated by increased broiler performance and nutrient digestibility in the present study. The xylanase supplementation also reduced ileal CD and increased intestinal VH. According to Mathlouthi et al. (2002), enzyme supplementation increases the mean villi height in broiler chickens. The lack of evidence on organ histological morphometrics could probably be due to the physical and chemical characteristics of the digested fiber in broilers chickens' digestive tracks. Several studies found that xylanase had no effect on intestinal morphology. However, Montagne et al. (2003) and Mateos et al. (2012) stated when broiler chickens were fed a diet high in fiber, fiber molecules were left behind in digestive villi, depending on the physico-chemical characteristics of the fiber in the diet, level of feed consumed, type of animal, the age and health status of the poultry. The effect of xylanase on the weight of proventriculus and CD of ileum also depended on the consumed feed types. Gonzalez-Ortiz et al. (2017) stated that the size of broiler chickens' internal organs and digestive tract is influenced by the type of feed given. Therefore, supplementing broiler chickens' ration with the high amount of fiber compounds influences the weight and morphometric traits of digestive organs because fiber can stimulate the physiological process of digestive organs mechanically and enzymatically.

CONCLUSION

The present study reveals the positive effects of xylanase supplementation on the performance and nutrient digestibility of broiler chickens. Broilers' body weight gain was increased and FCR value was reduced with the xylanase supplementation. However, the xylanase enzyme had no effects on the relative weight of organs (pancreas, gizzard, jejunum, ileum, and cecum) and the relative length of the cecum. Otherwise, xylanase supplementation reduced ileal crypt depth and increased intestinal villus height. Xylanase also interacts with decreasing FCR, increasing the digestibility of nutrients, namely fat, nitrogen, and phosphorus. In addition, it also interacts with reducing proventriculus size based on the type of feed. Xylanase interacts with decreasing FCR, increasing fat digestibility, and decreasing cecum size based on the sex of broilers.

DECLARATION

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Authors' contribution

Sri Rahmani Inayah contributed to data collection, database creation, and preparation of the manuscript. Yulianri Rizki Yanza was also involved in preparing the manuscript and data analysis. Rita Mutia, Anuraga Jayanegara, and Sri Amnah guided the research, data analysis, and manuscript preparation. All authors read and approve the final manuscript for publication in the current journal.

Competing interests

The authors declared that there is no competing interest.

Ethical consideration

All authors have checked the ethical issue, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

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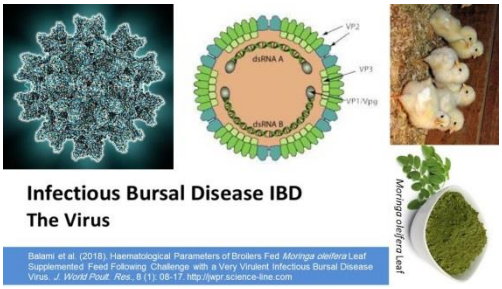

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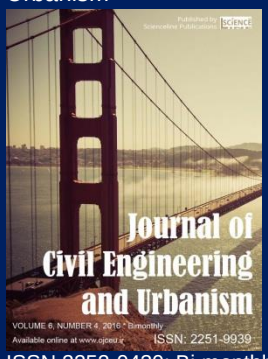

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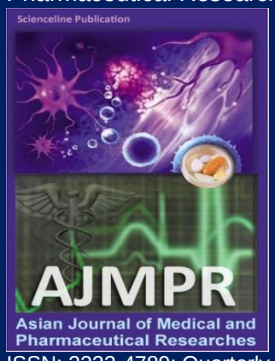

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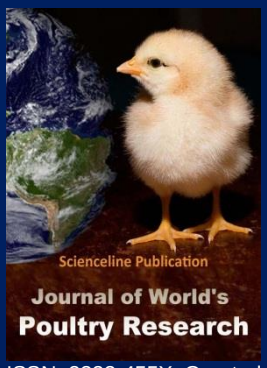

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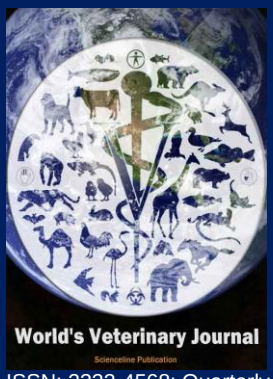

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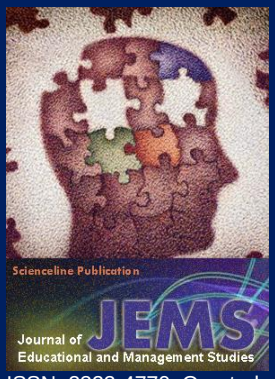

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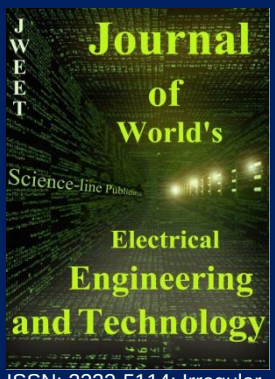

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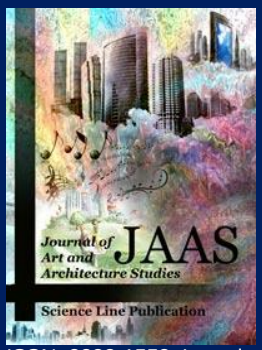

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